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Volume LXXXII Number 2

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The BOTANICAL GAZETTE

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OCTOBER 1926

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Volume LXXXII Number 2

THE BOTANICAL GAZETTE

A JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

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The PRESS IMPRINT

For September and October, 1926

Brains of Rats and Men

That man's brain has been slowly developed from other kinds of animal brain structures, that human behavior patterns and the noblest human faculties are functions of man's peculiar protoplasmic organization—to many these still seem difficult statements to accept. Their truth, however, is further demonstrated by C. Judson Herrick in *Brains of Rats and Men*, his new book on the origin and biological significance of the cerebral cortex. It is a volume that belongs to that rare class of scientific works of genuine literary merit, in which Mr. Herrick traces the evolution of this amazingly complex organ from fishes to men, and follows step by step the elaboration of its functions.

Objective behavior and subjective experience are here reformulated in biological terms. Mr. Herrick demonstrates incisively that the entire intellectual, emotional, and moral life of mankind can be explored as biological functions without loss of their supreme values as distinctively human attributes and without passing outside the realm of natural laws.

Others have tried to read human faculties backward into the lower animals. Mr. Herrick reverses the order and, beginning with the simplest structures prophetic of cerebral cortex, he reads this history through to its culmination in man. He outlines also the development of the thalamus and corpus striatum and their functional relations with the cortex.

He turns then to the mechanism of learning, centering the discussion about the brains of rats and men, the two species of mammals whose behavior patterns have been studied most intensively under conditions of laboratory control. Recent experiments upon learning in rats are summarized.

In considering the transition from brute to man, Mr. Herrick calls attention to the fact that the brain of man is more than twice as heavy as that of an ape of equal body weight and that this increase is chiefly in the cerebral cortex. The complexity of the internal texture of the human cortex surpasses that of an ape in still larger measure.

Mr. Herrick emphasizes the unscientific character of the oversimplification of human behavior so popular in some psychological circles. He believes that human nature cannot be understood without recognizing that its patterns of behavior are very different from those of rats and monkeys, and that conscious experience is an essential component of these human behavior patterns in their biological aspects.

Approaching the problems of human nature from the biological side and with biological technique, the author works out a common ground upon which objective psychology and introspective psychology may meet without sacrificing what is good in either method. He has demonstrated that, without transgressing the boundaries of biological science, we can set in their places objective behavior in all its manifestations: affect, impulse, volition, and the whole intellectual and moral life together with the structural mechanisms employed.

The concluding chapters have a special significance for psychology and education, since they throw light upon such problems as the subconscious, complexes, habit, vital reserves, experience, association, will, and the relations of mind and body.

This account of the cerebral cortex, its evolution and its functions, by one of America's foremost neurologists touches upon problems that are fundamental to all the sciences that take living things, and man in particular, as their province: biology, psychology, anthropology, the social sciences, education, medicine. *BRAINS OF RATS AND MEN*. By C. JUDSON HERRICK. *$3.00, postpaid $3.15.*

* * *

"Liberty, Equality, Fraternity"

Beyond all other ideas of government, democracy means not merely a governmental mechanism, but a way of life. The social philosopher who thus broadly conceives it must turn to the most general interests of mankind. Thomas Vernor Smith, in his new book *The Democratic Way of Life*, re-endows "Liberty, Equality, Fraternity"—worn slogans of a goal that has never been reached—with some of the spirit of the days when they were magic words; and makes a brilliant commentary upon the questions of humanizing work, the proper use of leisure, religion in a democracy, birth control, and democratic leadership.

To make clear the abiding real content of the democratic trinity of ideals, Mr. Smith inquires into these goals as they stand today. He looks upon liberty as fundamental, a natural end for life, and would restrict it only for the sake of fraternity. He admits that liberty is neither more nor less than doing as one pleases, but believes that through proper education men may please more and more to act fraternally.

Fraternity, Mr. Smith holds to be a natural and ethical end for man. It is, in his view, the basis of religion, and the function of religion in a democracy is to foster brotherhood within the group and universal brotherhood.

What can equality as an ideal mean in the light of the present-day facts? Mr. Smith's equalitarian ideal means fundamentally that whatever objects or activities are regarded as good must be admitted to be as good when experienced by one person as when experienced by another. He sees in war and industrial autocracy the arch-enemies of equality, and in the cessation of wholesale hostility, the development of industrial democracy, necessary measures for the attainment of the equality ideal.

Bertrand Russell has expressed the belief that with present technology, the four-hour day awaits only the will to have it. Regarding such optimism as at best prophetic of a distant time, Mr. Smith, in considering work and leisure, prefers to place emphasis upon the humanizing of work, and to give up the tenaciously held idea that the good life is to be found exclusively in leisure. The supreme moral task of the hour, in Mr. Smith's view, is in banishing drudgery and monotony, and investing otherwise unpleasant work with meaning.

What kind of leadership is needed in a democracy? Mr. Smith rejects the conception of leadership of prestige and authority inherited from monarchies, and replaces it with the new conception of leadership suggested by science. The new type of leader is a leader by virtue of knowledge, and his function is to point, not to order. Science having discredited the spectacular and omniscient type of leader, democracy must be judged not by its ability to produce a few "master-leaders," but by its ability to make every citizen a creative leader in some enterprise.

Mr. Smith has brought to these principles of democracy, upon which so much enthusiasm has been lavished and over which so much disappointment has been felt, an undeniable tinge of modernity. He has formulated a democratic credo, a profession of the social faith of a modern man. *THE DEMOCRATIC WAY OF LIFE*. By THOMAS VERNOR SMITH. *$1.75, postpaid $1.85.*

A New French Grammar

One hears it frequently said that of the making of French grammars there is no end but that the French grammar has yet to be written. That is to say a new one may legitimately make its bow without regarding too much the weight of its predecessors.

The new *Introduction to the Study of French* by Otto F. Bond will make a place for itself among French texts where the rapid attainment of the ability to read and understand French is the end most desired. Teachers who find that the prolonged syntactical study and delayed actual reading made necessary by most grammars is somewhat boring both to instructor and student will welcome this grammar which equips the student in twenty lessons to read interesting material.

It is a "comprehension" grammar, brief, thorough, and intensive, and written from the point of view of the student struggling with the difficulties of the printed page and the spoken word and desiring a mastery of these problems early in the course.

The "reading method" upon which this book is based has been in use in the Junior College of the University of Chicago for five years with remarkable success. It has led to an average reading of 1,500 to 1,800 pages in the first year of instruction in French.

In an article in the *Modern Language Journal* for April, 1926, Mr. Bond says: "If Mary White wants to read *Le Petit Chose* or *Abeille* before she can order from a French menu or juggle a pair of partitive constructions in a negative sentence, why not let her do it? She cannot fail to get some good out of the performance even if she doesn't get all the story with full literary appreciation. Suppression of her desire to read cannot be expected to heighten her interest in partitive constructions!"

In preparing a text which concentrates on the problem of getting the student to the point where he can follow his natural interest in reading, Mr. Bond has performed a valuable service for instructors whether or not they agree with him that the primary aim of the junior college course should be the development of reading ability. The text may be followed by the direct method or the grammar-translation method, and its value as a starting-point for the course and a means for creating interest in the course is not lessened.

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THE
BOTANICAL GAZETTE

October 1926

ORGANIZATION AND SIGNIFICANCE OF LENTICELS IN DICOTYLEDONS

II. LENTICELS IN RELATION TO DIFFUSE STORAGE RAYS OF WOODY STEMS[1]

RALPH H. WETMORE

(WITH PLATES VII–X)

In a previous article, cauline lenticels were shown to be related definitely to storage rays of the aggregate and compound type. Moreover, the type, size, and distribution of the lenticels on the stems of these forms were shown to reflect the internal organization of the radial storage structures within. In contrast with the relatively few species with aggregate or compound storage organs, the greater proportion of our trees and shrubs possess diffuse storage rays, although the diffusions are of extremely varying extent. It is more general for the original storage aggregations to be broken up into multiseriate units of one to four cells in width. Representative of this are fig. 2, of *Prunus pennsylvanica*, and fig. 10, of *Cephalanthus occidentalis*. In contrast with these, other forms do not have the diffusion carried to such an extreme degree, and the multiseriate entities are 5–12 cells wide. Such a condition is apparent in *Fagus grandifolia* (fig. 14) and *Kalmia latifolia* (fig. 17). Of course all transitions occur between these two groups; there is clearly no line of demarcation. Since the forms of diffuse rays are so numerous, however, the writer has taken this arbitrary method of grouping, in an endeavor to show that the nature of the lenticular structures is corre-

[1] Contribution from the Laboratories of Plant Morphology, Harvard University.

lated intimately with the character of the ray. No idea of permanent classification or grouping is intended thereby, the division being but a matter of convenience. In an equally arbitrary manner, the writer has divided the first group again into those in which the rays remain the same size in the phloem as in the xylem, as was shown in the photographs of transverse sections of *Prunus pennsylvanica* (fig. 2) and *Cephalanthus occidentalis* (fig. 10), and those in which the multiseriate rays tend to become V-shape in the phloem region, as is apparent from fig. 27, *Tilia americana*, and fig. 30, of *Carya ovata*.

Type I. Diffuse rays 1-4 cells wide in both xylem and phloem

In these forms, with diffusions of one to four cells in width in both xylem and phloem, we find representatives with transverse and others with longitudinal types of lenticels, as in previous groups. The species of *Betula* and most species of *Prunus* are exceedingly good examples of the transversely oriented structures. Fig. 2 is a photomicrograph of a transverse section of *Prunus pennsylvanica*. Here the horizontally elongate lenticel is distinctly confronting many rays. In this form the pericycle is practically continuous, although occasional thin places and even breaks are found to exist over the rays. Due to the low magnification necessary to include the whole lenticel in the field, this detail is unavoidably indistinct. An interesting condition is apparent in the phloem. The rays seem flanked on one or both sides by lacunae which extend almost down to the cambium. From the point of view of aeration this would be especially valuable. Such a feature is not uncommon and is especially characteristic of all species studied in this genus. The ray cells seem to grow so much larger than the adjacent phloem cells that they are torn away from the latter on sometimes one, sometimes both flanks. The different size of the ray cells in xylem and phloem is apparent, even with this magnification. In the xylem the cells are so small comparatively that no cell structure is visible at all. On the other hand, the cells of the phloem ray are clearly visible.

A study of such sections raises again the question of an anatomical explanation for the transverse orientation of the lenticels. Remembering that in the conifers it was correlated with short rays,

it seemed advisable to note the situation here. Fig. 6 is a tangential view of the wood of *Betula alba* var. *papyrifera*, taken from near the cambium. The multiseriate rays are clearly short, measuring between 0.15 and 0.5 mm. Similarly a tangential view of *Prunus pennsylvanica* (fig. 5) shows an analogous condition in the radial structures. They are slightly wider, averaging three or four cells, but in height they vary between 0.4 and 0.7 mm.

To contrast these conditions with those of stems possessing longitudinal lenticels, the situation in *Prunus virginiana* was studied. It is interesting that *Prunus*, like many other genera, possesses both kinds of lenticels. The majority of the species are as in *P. Cerasus* (fig. 1) and *P. pennsylvanica*, but *P. Padus*, *P. virginiana*, and *P. melanocarpa* show lenticels of longitudinal nature. Any contrasting features between *P. pennsylvanica* and *P. virginiana* should therefore be especially instructive. Figs. 4 and 8 represent transverse and longitudinal sections respectively of this species. Here the single lenticel shown in the first photomicrograph is distinctly subtending a single multiseriate ray. Such rays, as in *P. pennsylvanica*, are flanked by lacunae, a result again apparently of unequal growth of ray and phloem cells. Also, although not visible at the magnification of the photograph, the pericycle is discontinuous and sclerotic, with the interruptions under the lenticels. In tangential section, taken under the same magnification as that of *P. pennsylvanica*, the larger rays appear distinctly longer, measurements revealing them up to 1.5 mm. in length.

In this connection *Cephalanthus occidentalis* (Rubiaceae) is worthy of note. The lenticular conditions are visible in the segment of the stem pictured in fig. 9. Here the large lenticels, especially numerous below the nodes, are clearly longitudinal. Fig. 10 indicates the conditions in the transverse section, and fig. 12 in the tangential view. The former shows that the lenticel is not related to one of these rays, but to many, since the rays are very small and numerous in their diffusion, being but one and occasionally two cells wide. The longitudinal section provides a key to the nature of the lenticel. These rays, although narrow, are not short. They run, in some cases, the whole length of the section; in general, however, they average 2.5–3.5 mm. Moreover, a very much disorganized pericycle is ap-

parent here. The cells can be seen in small groups of two or three each. This condition must facilitate aeration to a very considerable degree.

Many more examples, equally illustrative, exist of this principle. In every case examined, the crucial factor seemed to be the vertical height of the diffuse rays. If the rays are comparatively short, the lenticels are transverse; if longer, they are longitudinal. In general, whatever the condition of the pericycle might be in transverse section, whether continuous or slightly broken, it was usually more disorganized in forms characterized by the longitudinal lenticel. Many other instances of genera like *Prunus* might be given, where both types of lenticels exist, such cases lending themselves exceedingly well to a study of comparative conditions. For example, *Ilex*, *Rhus*, and *Sorbus* serve well this purpose. *Ilex verticillata* has excellent transverse lenticels; *Ilex glabra* possesses longitudinal lenticels. In the former the multiseriate rays average 0.2–0.4 mm. long, whereas in the latter they have a mean extension of 0.7–1.5 mm. In *Rhus*, *R. Vernix* has transverse lenticels, and the diffuse rays in no case measured more than 0.6 mm. in height. On the other hand, *R. typhina* and *R. toxicodendron* have longitudinal lenticels, the larger rays in the former averaging about 1 mm. in height, and in the latter often reaching a length of 1.2 mm. In *Sorbus* a comparable situation exists. *S. domestica* has horizontally elongated lenticels, with multiseriate rays measuring 0.1–0.35 mm. in vertical extension; *S. americana* and *S. intermedia* show small longitudinal lenticels, while the rays in these two species are longer, running up to 0.6 mm.

Many more isolated examples can be given. Thus *Ligustrum vulgare* has transverse lenticels and rays averaging about 0.5 mm. in height. On the other hand, *Rhamnus cathartica*, *Pyrus melanocarpa*, *Maackia amurensis* var. *Buergeri* all have longitudinal lenticels, and rays averaging 0.5–1.0 mm. in height. These examples lead to no absolute results, in the sense that below a certain height of ray the lenticels are transverse, and above that height they are longitudinal. Certainly this is not the case. In fact, the evidence might be considered conflicting except when considered in the light of genera where the two types of lenticels coexist. Here the comparative

status of the problem becomes apparent. One cannot examine numerous sections of many of these species, of which only a representative number have been referred to here, and not be impressed with the fact that the relationship is a real one. In these genera those forms which in the diffusion of their foliar rays have gone farthest, producing bands of tissue but few cells high and few wide, are best aerated by transverse lenticels which subtend many of these comparatively short rays. Where the diffusion of the rays results in multiseriate entities of longer dimension, the longitudinal lenticel will obviously more efficiently aerate the structure. If the rays are very narrow, indicating that the diffusion has gone very far in this direction, the lenticels may be broad as well as long, as for example *Cephalanthus occidentalis*, and thus many may be aerated by one lenticular structure. In such a case the lenticels are usually fewer in number. On the contrary, if they are small, each confronting a single ray, they are invariably more numerous. This relation between the size of the lenticels and their number was shown by DEVAUX (1). Isolated species studied are of little value in solving the problem, for there are no others to compare them with definitely; but once a conclusion is reached from the genera permitting an actual contrast of conditions, because of both types being present, the relationships in others can be understood. If one examines the lenticels and finds them transverse, the storage rays will be found relatively short. Conversely, if the lenticels are longitudinal, these rays will be comparatively longer. In no case did the writer find outstanding exceptions to this generalization. Moreover, a secondary factor was found in the sclerotic pericycle. It is rather an exception than a rule to find a continuous pericycle with longitudinal lenticels. Usually, if not disorganized into nests of cells of varying numbers, it is at least interrupted under the lenticel itself. Thus in general again, the lenticular device seems that best correlated with the internal anatomy to provide gas exchange efficiently to the particular type of ray in each individual case. Although uniseriate rays are present, their part is seemingly, as STRASBURGER (4) claimed, but an adjuster of sap flow throughout the stem. At no time did the writer find intercellular spaces in these structures.

Type II. Diffuse rays more than four cells wide in both xylem and phloem

In the stems possessing larger diffusions, internal conditions in general are much as in those of the preceding type. However, two additional features worthy of note early became apparent. The first of these is the scarcity of forms possessing transverse lenticels. In all the species examined, but two of these were located (*Ribes alpinum* and *R. multiflorum*). All others, and there were many, bore longitudinal aerating structures.

The second feature was the scarcity of arboreal representatives having this type of diffuse ray. All but three (*Fagus grandifolia*, *Oxydendrum arboreum*, and *Parthenocissus tricuspidata*) were shrubs. Parallel to this situation was another, that all of these fruticose forms with wider rays were discovered to have the initial periderm deep seated; that is, it originated within the pericycle, in immediate contact with the phloem. In this way epidermis, cortex, and pericycle were early lost, and the lenticels existing in this periderm were therefore in immediate contact with the stele. STAHL (3) first recognized this last fact, but treated it as of no immediate advantage, except that it afforded him definite proof that lenticels might arise directly from phellogen as well as under stomata. He referred to this situation as being a very common one, and listed as examples, among others, *Berberis*, many species of *Spiraea*, and various species of *Ribes*. DEVAUX (1) also mentioned these deep seated lenticels. The fact that this situation is so characteristic of shrubs seems one worthy of note, and especially so since it is primarily a characteristic of only those shrubs possessing wider diffuse rays. Not a single example was found that showed a periderm of deep origin, and at the same time diffuse rays but a few cells wide. In this connection it is instructive to know that very few of the trees examined possessed the wider type of diffuse ray. Naturally, wider sheets of parenchymatous tissue would be disadvantageous from a mechanical point of view in arboreal forms. Moreover, in their rhytidome and secondary bark formation, trees possess the equivalent of the deep seated periderm of shrubs. In contrast with the larger forms, shrubs can possess wider rays without serious mechanical disadvantage. With their smaller, slow growing stems and branches they can

lay down an initial periderm within the pericycle, and thus complete at once what the tree accomplishes ultimately only through a series of secondary periderm formations.

Considered from the point of view of aeration, deep seated lenticels become very significant. Primitively the lenticels were separated from the central cylinder by both cortex and pericycle. It is true that these possessed intercellular spaces, a generally recognized fact, making aeration possible, but the absence of pericycle and cortex would certainly facilitate gas exchange very materially. This is apparent in the tendency toward a discontinuity in the pericycle, especially under the lenticels of the longitudinal type. The loss of both pericycle and cortex seems a logical step in nature's endeavor to increase this vital activity in accord with the needs of the plant.

As illustrating the situation in the two arboreal forms which have larger diffuse rays, it will be well to refer to *Fagus grandifolia*. It is worthy of note that this form possesses a superficial periderm. Fig. 11 indicates an external view of a portion of a branch of this species. The lenticels here are longitudinal, small and scattered. Fig. 14 portrays a transverse view, showing the large diffuse rays, which average 8–10 cells in width. Opposite one of these is a lenticel. Such was invariably the situation in this species, lenticels always subtending these larger rays. It is interesting that STAHL (3) refers to a similar situation in *Platanus*, although no importance was attached to the fact. In *F. grandifolia*, it should be mentioned also that interruptions in the otherwise continuous pericycle are commonly found under the lenticels. *Oxydendrum arboreum* is an arboreal ericaceous form and is also deserving of attention, since it is the only member of this family that the writer found to possess superficial periderm. The rays here are long vertically, and are frequently arranged in longitudinal rows, so that one can understand the presence of longitudinal lenticels.

As stated, by far the greater number of species of type II have a deep seated periderm. The only two forms of this group with transverse lenticels disclosed by these investigations are *Ribes alpinum* and *R. multiflorum*. Fig. 13 indicates the external condition of *R. alpinum*. The transverse lenticels are very prominent and extend a considerable portion of the way around the stem. Internally, it is

very significant that the multiseriate rays are short, even though they are much wider than usual (fig. 15). In height they average 0.6–0.8 mm. This correlation of orientation of lenticel and height of ray is again remarkably in accord with the other results of this research. *R. multiflorum* proves a similar case.

In contrast with these two forms with transverse lenticels are those possessing longitudinal lenticels. Here, as has been mentioned, belong a great many of our ordinary shrubs. All fruticose members of the Ericaceae examined (twenty-one species in all) were of this type. Here also are included the ten species of *Spiraea* examined. *Berberis vulgaris* and *Lonicera ovalis* are further common examples as well, and many others might be listed.

Kalmia latifolia illustrates conditions in the Ericaceae. Fig. 16 shows a portion of the stem, and fig. 17 a cross-section of the same. The former clearly shows the long vertical lenticels, resembling grooves. The latter equally as clearly connects these lenticular grooves with the ends of the multiseriate rays. The illustration permits one to see that the cortex and pericycle are gone, and that the periderm of alternating layers of cork and phelloid is continuous except at the lenticels. These are indented, as are the annual rings in the region of the rays, and by reason of the minimal amount of complementary tissue present, they might not be considered as lenticels. Their location and correlation, however, show them undoubtedly to be lenticular in nature. Tangential sections prove that the larger rays, occurring under the lenticels, are of considerable depth vertically. In view of the length of the lenticels themselves, this situation is significant.

In the Ericaceae there were sectioned and studied thirteen species of shrubs. In every one of these the same situation is found, except that the lenticels usually are not so elongated vertically. In all cases the secondary longitudinal lenticels are related to multiseriate rays of varying width, which often reach a height to be measured in millimeters, and not in fractions of a single millimeter. Another feature is characteristic of the greater number of these forms. On reaching the phloem the rays immediately broaden so that the outer ends of these structures confronting the lenticels are many times wider than the inner ends in contact with the xylem. In this way a greater surface of the ray is exposed, thus facilitating aeration.

The situation in the various species of *Spiraea* is especially instructive. The modes of distributions of the lenticels are striking, as they differ to such an extent in the different species. But three examples will be used here as illustrative of the group. Fig. 18 indicates the situation in *S. Veitchii*. A nodal or leaf scar is visible near the top of the section. Below this on either side a definite row of lenticels can be seen, these rows continuing downward for a considerable distance. A few small lenticels can be detected elsewhere on the stem. This general situation is true for every node, after the cortex and pericycle have been exfoliated through the periderm formation. In fig. 21 can be seen three illustrations of *S. gemmata*. Here the lenticels are few in number and grouped around or near the node, occasionally running down directly below it, in one median cluster, for a short distance. Fig. 19 represents a short portion of *S. salicifolia*. Here a single row of lenticels is found above the node, while below this point there are two flanking lines of these structures extending downward for a considerable distance. Other species with their characteristic lenticular arrangements might be indicated as well, but these will serve as types. The clue to the problem of these variable distributions is furnished by the tangential sections. Fig. 22 is such a view of *S. Veitchii*. Below the leaf trace the ray quickly becomes diffuse, breaking up into multiseriations of varying widths. The interesting part is that these multiseriate units are uniformly distributed, except on either side below and lateral to the trace, where a row of these rays is found running vertically in the stem. The larger size of the components of these rows makes them very distinct. Could more of the tangential section have been shown, they would have been found to extend a considerable distance downward, until in proximity to another trace. The situation in *S. salicifolia* is very similar below the node, the lateral rows of multiseriate rays being very pronounced (fig. 23). Extending above the node can be seen a part of the undivided portion of the ray. The flanking lenticels below the trace and the single row of lenticels above it thus seem to have distinct internal relationships. A similarly oriented section of *S. gemmata* is shown in fig. 24. Here it is evident that the grouped rays separate quickly, and a short distance below the node no lateral rows are apparent; in fact, the groupings so quickly become diffuse that the storage aggregation soon cannot be differenti-

ated from those in the remainder of the field. In this form it will be remembered that a cluster of lenticels is located around the node, being apparent below this point for only a very short distance. In other species of *Spiraea* the lenticels are as uniformly scattered just below the leaf scars as elsewhere. In these cases scarcely a sign of a concentration of multiseriations near the node is apparent in tangential section, so quickly do they become diffuse.

An epitome of this situation seems to be found again in the correlation of ray and lenticel. This is very strikingly illustrated in *Spiraea* and the Ericaceae. Whatever specific type of diffusion exists in the storage ray, it is closely paralleled by the superficial distribution of lenticels. Still further is the aeration facilitated by the early loss of epidermis, cortex, and pericycle. The fact that the multiseriate structures frequently lie in vertical lines also explains the equally frequent corresponding vertical rows of lenticels. The concentration of lenticels below the nodes, as in *Spiraea*, has been noticed by earlier botanists. TRÉCUL (5) referred to the occurrence of a half-crown of lenticels below the leaf insertion in *Ficus Carica* and some willows. DEVAUX (1) mentions that the only lenticels found in *Daphne* and *Veronica* are localized in places in the neighborhood of the leaf scar; and other forms, like *Ailanthus glandulosa*, *Sambucus nigra*, *Aralia Sieboldii* (*Fatsia japonica*), etc., have them more conspicuously in the nodal region than elsewhere. When they do exist, he claims, they exist in pairs, one on either side of the axillary bud. Certain herbaceous forms have a number of lenticels on the actual leaf scar, as, for example, *Malva rotundifolia*, *Melilotus macrorhiza* (*M. officinalis*), *Brassica nigra*, etc. In summary, he states: "Il existe donc aux noeuds spécialement au-dessous de la feuille, des conditions internes persistantes, qui ont dès le début une grande influence sur le développement lenticellaire, quelle que soit l'origine des lenticelles." Since he was explaining lenticels in terms of transpiration, "des conditions internes persistantes" completely evaded him. However, specific instances of relationship between lenticels and rays have been noticed in the past. Thus SCHENCK (2) mentioned that the larger rays of *Aeschynomene sensitiva* were in direct connection with the lenticels, and were so aerated. STRASBURGER (4), although he quoted SCHENCK, gives no specific cases, but he does say

in general that the rays are aerated directly from the lenticels through the aeriferous cortex. It seems from the many forms studied by the writer that any such relationship of the lenticels to the nodes is due to their proximity to storage rays within. Whether there is one on either side, as DEVAUX reports in *Daphne* and *Veronica*, and as the writer has commonly found in *Berberis vulgaris*, *Parthenocissus tricuspidata*, etc., or whether a circle exists below, as TRÉCUL (5) noted in *Ficus Carica*, or whether varied conditions exist as in *Cephalanthus occidentalis* or *Spiraea*, there is seemingly a direct correlation with the type of ray within. STAHL (3) mentions *Abies pectinata* as having nodal lenticels. The situation here, however, is very different.[2] The nodal relationship in the conifers is a primitive one, due essentially to the presence of an aerating device, the parichnos, in at least the more primitive forms. In the angiosperms the grouping near the node is of different significance. The lenticels are now related to the storage elaborations instead of to the traces themselves as in the conifers. There is also the tendency in these forms with wider diffusion for the multiseriate structures to be longer vertically, either as a result of the individual rays not being broken up into shorter units, or else by the retention of these in longitudinal rows. In these higher forms, therefore, the lenticels accordingly are often very preponderantly extended vertically. Thus, one might say that in the evolution of the lenticel, the transverse type is being replaced by the longitudinal in these forms, for thereby the physiological need of gas exchange is much more readily supplied. Moreover, not only single longitudinal lenticels, but often vertical rows of these are present, thus insuring the aeration of the correspondingly situated rays within.

Type III. Diffuse rays 1-4 cells wide, tending to become V-shaped in phloem

As stated earlier, the diffuse rays present in angiospermous trees are almost invariably only a few cells wide. These rays of narrow dimensions, however, usually tend to become very much wider in the phloem, until they are pronouncedly V-shaped. In this way the

[2] JEFFREY, E. C., and WETMORE, R. H., Ann. Botany. Unpublished.

rigidity of the tree is not lessened to any extent, while the provision for increased aeration is magnified many times.

It is interesting that certain shrubs possess indications of this situation. Thus it was mentioned that practically all fruticose Ericaceae studied showed a tendency for the rays to become wider in the phloem, especially in close proximity to a lenticel. *Myrica asplenifolia* offers an instance of the same thing happening under lenticels only; however, the situation is not common nor characteristic in shrubs. It is only in arboreal forms that we can see a complete series indicating how this so-called V-shaped phloem ray has its origin, and to what degree it has evolved in some forms.

Salix provides a noteworthy primitive condition in these phloem rays. Fig. 25 shows a transverse section of *Salix alba* var. *vitellina*. Here the ray under the lenticel is clearly V-shaped, although not starting to fan out until some distance from the cambium. On either side can be seen a single instance of the same phenomenon, but to a less marked extent. In the xylem all diffuse rays look alike, but all of these do not become V-shaped in the phloem. The pericycle opposite those which do becomes discontinuous, and is found only over the phloem groups, although this is not apparent at the magnification of the photograph.

The situation in *Carpinus cordata* is equally instructive. It is interesting to note that in this genus certain species have aggregate storage rays (*C. caroliniana* and *C. japonica*), while *C. cordata* possesses the diffuse type. In this species were observed (fig. 20) the largest lenticels discovered. A transverse section (fig. 26) shows the internal situation. The lenticels are remarkably broad for those of longitudinal nature, covering a large number of diffuse rays. However, the figure shows that but a small number of the latter become V-shaped. It is worthy of note that the pericycle becomes disorganized opposite these rays, bringing about a close connection between the lenticel and the V-shaped rays. That this pericycle is continuous elsewhere should be mentioned. These phloem rays become of considerable length and clearly abut on one another when seen in tangential view, so that the large lenticels obviously constitute an advantageous correlation. It may be pointed out, however, that smaller lenticels in greater number would subserve the same purpose as well. This will be apparent in the following examples.

Benzoin aestivale (fig. 3) is definitely of a higher type. Here the larger rays all tend to become V-shaped in the phloem, due to meristematic activities in the rays themselves. The phloem masses alternating with these rays are all clearly subtended by sclerotic nests of the discontinuous pericycle, but none of these cell groups appear opposite the rays. Not all of the rays have lenticels confronting them in this particular section, but serial sections indicate that such an organ exists for practically every one. Fig. 7 illustrates the great number of these small lenticels, and the tendency for them to become arranged in rows. The multiseriate rays in the xylem vary between 0.2 and 1.1 mm. in height, but in the phloem they average about 2 mm. This increase in length of rays in the phloem is a character frequently found, but especially so with those which become V-shaped. It is this situation that enables one to explain the vertical rows of lenticels.

In *Tilia americana* (fig. 27) the cross-section shows practically every multiseriate ray with the tendency to become V-shaped, although in varying degrees. This is brought about by definite anticlinal divisions in the cells of the rays, as is apparent from the tangential view in fig. 28. Here the cells of the rays are clearly in transverse periclinal rows, which would be possible only by divisions in an anticlinal direction. One is not able to identify any definite meristem, however, any and all cells seemingly being capable of division. That this is true is easily discernible under greater magnification, for cells of all sizes are apparent, some being recent and some older products of division. This tangential section distinctly indicates the great length of the rays in the phloem. In the xylem the longest ray measured was 2 mm., while in the phloem they varied between 3 and 4 mm. In accord with this fact, there is present again a tendency for the lenticels to be distributed in vertical rows of two or three, as shown in fig. 31, a superficial view of a portion of a branch of *T. americana*.

Of a nature still more striking, although similar in several respects, is the situation in *Carya ovata*. Fig. 30 is a transverse section showing the large V-shaped rays, and the intervening pointed phloem masses capped with nests of pericycle cells. The lenticels in this species are very small, but over each of the three phloem V's shown is a lenticel, indistinctly visible. Extending between the

bottom of each of these V's and the lenticular organ, there is distinctly apparent a light area, visible in both ray and cortex. The situation here noticeable is much more distinct in fig. 32. This represents a portion of one of the V-shaped rays in tangential view, under higher magnification. There is evident from top to bottom a definite meristem, the cells on either side being remarkably seriate. The light appearing areas visible in transverse section, therefore, are regions of cambial activity. That the cortex should assume these divisions as well is of additional interest. The height of the rays in *Carya ovata* offers an interesting study. In the xylem no multiseriate ray was found which measured over 1.3 mm. In the mid-phloem region, a maximum length of 6 mm. exists for these rays. In striking contrast with this, just within the pericycle the rays measure 9–14 mm. In other words, the rays increase from seven to ten times in length in the phloem. This is truly remarkable, and must indicate transverse anticlinal divisions as well as vertical. The lenticular situation accompanying these internal highly evolved structures is very instructive. Fig. 29 is a portion of a branch of *Carya ovata*. The vertical rows of small lenticels are undoubtedly like those seen in the transverse section. Moreover, removal of the bark shows that they are directly over the large rays. The lenticels in these rows are so approximated that they almost constitute a single very long structure. The fissures from lenticel to lenticel are practically continuous. It is not surprising, therefore, that the identity of these small lenticels is entirely lost in the elongated structures of *Amelanchier oligocarpa* (fig. 33). This type of lenticular relationship with V-shaped phloem rays is comparatively common in trees of various affinities, as *Carya glabra*, *Castanea dentata*, *Liriodendron Tulipifera*, *Fraxinus nigra*, etc.

In summary, the V-shaped ray in the phloem is a structure which originates through active cell division of the initial multiseriate ray. Although usually these divisions are diffuse, they may in certain forms become so localized as to form a definite cambium extending the entire radial length of the ray, and even across the cortex to the lenticel. Also, as a result of other divisions, the ray increases in length as well as in width, until a very broad front is in juxtaposition to the lenticel. As a result of these meristematic activities, the peri-

cycle becomes broken up into nests which are localized at the peaks of the phloem masses. The lenticels are all longitudinal, are invariably opposite the phloem V's, and in general are arranged in vertical rows, limited more or less in length by the extension of the ray within. Although occasionally large, they are usually small and numerous, and frequently so close together that with age the individual limits are lost and the structure assumes the appearance of a single monstrous complex.

The advantage in the evolutionary development of this type of structure in trees is not difficult to picture. In order to give the maximum rigidity to these arboreal forms, so that they might successfully withstand the winds, the uniformly diffuse storage ray of small dimensions has evolved. In this way the stele is interrupted to a minimal amount in any one place by living parenchymatous tissue. Then to supply the maximum storage so necessary to the efficiency of these angiospermous forms, the V-shaped phloem ray has evolved. Here it in no way impairs the mechanical rigidity of the stem in relation to violent stresses and strains. Moreover, it has an additional advantage in that the major part of the storage tissue is placed near the periphery of the stem, thereby greatly increasing its chances for aeration. Originally it seems that only those rays nearest the lenticels assumed meristematic activities and became V-shaped. In higher forms like *Tilia americana*, *Carya ovata*, etc., practically every multiseriate ray assumes this form in the phloem, and associate vertical lines of lenticels are found to exist with each. As a consequence, one cannot help but think of the evolution of storage rays in the phloem of the cylinder as closely correlated, to a great degree at least, with the function of aeration. This is borne out in a most striking manner by the external evidence supplied by the arrangement of the superficial aerating organs, the lenticels. The funnel-like nature of the rays in the phloem facilitates the aeration of the living elements of the stele.

The only reference which the writer has found to these V-shaped rays is that of STRASBURGER (4). He mentions these structures as possessing intercellular spaces in *Tilia*. This condition has been confirmed time and again in many species by the writer. The literature seems to furnish no reference to the generally diffuse meristematic

development in the forms discussed here, nor of the presence of an actual cambium in *Carya*.

Discussion

The study of forms possessing the three types of diffuse rays, as artificially divided in this research, results in at least one very evident conclusion. There seems to be no doubt of a very close correspondence between the elements of the foliar ray and the lenticular structure evolved for its aeration. This correlation is so apparent that a superficial study of the lenticular devices enables one to predict with considerable accuracy the character of the rays which they cover; and vice versa, study of the transverse and longitudinal sections permits one equally well to be reasonably certain of the type and distribution of the lenticular organs which subtend them.

It is of interest to note that forms with transverse lenticels are decidedly in the minority among those species possessing diffuse storage rays. Generally only those more primitive trees and shrubs which have retained the narrow, short, diffuse rays, without modification, possess these structures. Usually, wider multiseriations are at once correlated with the longitudinal lenticel. Thus we find *Spiraea salicifolia* and others possessing lines of longitudinal lenticels confronting lines of vertically elongated, large, multiseriate rays; and in *Kalmia latifolia*, single, greatly extended longitudinal lenticels corresponding superficially to each immensely long ray. Still further is this type of lenticel advantageous when the rays in the phloem acquire meristematic activities and increase not only their length, but their transverse dimension as well, as exemplified in the V-shaped rays of this part of the stele. Here again not only single longitudinal lenticels, but rows of them appear, as in *Carya ovata*, *Amelanchier oligocarpa*, etc. It is thus fitting to conclude that the longitudinal lenticel represents the greatest evolutionary advance, and naturally, therefore, the internal structures accompanying present the greatest range of variability.

Summary

1. Lenticels occurring on shrubs and trees possessing diffuse storage rays are directly related in position, structure, and size to those

rays, just as they were in cases of forms with aggregate or compound rays. In this way the aeration of the rays and other living tissues is affected.

2. Transverse lenticels exist much less frequently on forms with diffuse storage rays than do longitudinal lenticels. When present, they are universally associated with vertically shorter storage rays.

3. Longitudinal lenticels are more characteristic of woody forms with diffuse storage rays. In these cases the rays are always longer vertically. Frequently they are very long or they are arranged in vertical rows. At such times the rays are confronted by greatly elongated lenticels or vertical rows of lenticels.

4. Some trees, as *Tilia*, *Carya*, *Fraxinus*, possess V-shaped rays in the phloem. These rays become greatly elongated as well. Such increases in width and length are brought about by meristematic activities in the phloem region of the ray. These broad rays are confronted by rows of lenticels, thus facilitating their aeration.

5. The general existence of a deep seated periderm in shrubs and of a rhytidome in trees makes possible a still more intimate connection between the lenticels and the rays within.

6. The organization and evolution of the storage rays in the stems of angiosperms has been accompanied by a parallel organization and evolution of the lenticels. In the root, however, the more primitive transverse lenticels in their original relationship to the appendages have been retained. This is in accord with the conservative nature of the root.

In conclusion, the writer wishes to express his appreciation of the criticism and advice of Professor E. C. JEFFREY during the course of this research.

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EXPLANATION OF PLATES VII–X

PLATE VII

FIG. 1.—Portion of trunk of *Prunus cerasus* with horizontally elongated, transverse lenticels; $\times\frac{1}{8}$.

FIG. 2.—Portion of transverse section of *Prunus pennsylvanica*, showing relation of transverse lenticel to several multiseriate rays; $\times 25$.

FIG. 3.—Portion of transverse section of *Benzoin aestivale*, with V-shaped rays in phloem; $\times 25$.

FIG. 4.—Portion of transverse section of *Prunus virginiana*, showing relation of longitudinal lenticel to single multiseriate ray; $\times 25$.

FIG. 5.—Tangential section of *Prunus pennsylvanica*, showing height of multiseriate rays; $\times 40$.

FIG. 6.—Same for *Betula alba* var. *papyrifers;* $\times 40$.

FIG. 7.—Portion of stem of *Benzoin aestivale*, showing lenticels; $\times 1$.

FIG. 8.—Tangential section of *Prunus virginiana*, showing height of multiseriate rays; $\times 40$.

PLATE VIII

FIG. 9.—Segment of stem of *Cephalanthus occidentalis*, showing lenticels; $\times 1$.

FIG. 10.—Portion of transverse section of *Cephalanthus occidentalis*, showing relation of large longitudinal lenticel to many diffuse rays; $\times 27$.

FIG. 11.—Portion of branch of *Fagus grandifolia*, showing lenticels; $\times 1$.

FIG. 12.—Tangential section of *Cephalanthus occidentalis*, showing great length of rays; $\times 40$.

FIG. 13.—Segment of stem of *Ribes alpinum*, with very prominent transverse lenticels; $\times 1.5$.

FIG. 14.—Portion of transverse section of *Fagus grandifolia*, showing relation of lenticel to a large ray; $\times 27$.

FIG. 15.—Tangential section of *Ribes alpinum*, showing short wide rays; $\times 40$.

FIG. 16.—Segment of stem of *Kalmia latifolia*, with very long longitudinal lenticels; $\times 1.5$.

FIG. 17.—Transverse section of same, showing relation of lenticels to multiseriate rays; $\times 10$.

PLATE IX

FIG. 18.—Segment of stem of *Spiraea Veitchii*, with lenticels in two flanking lines below node; $\times 1.5$.

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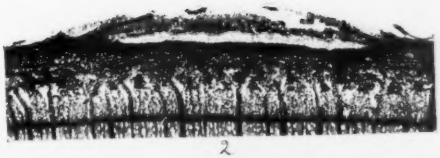

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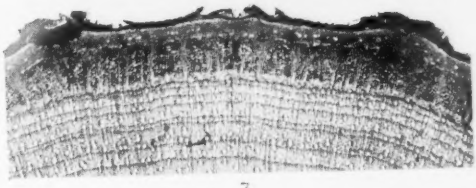

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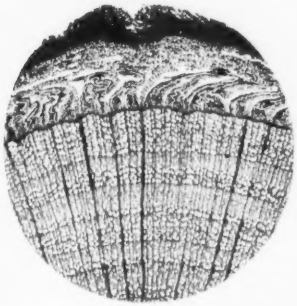

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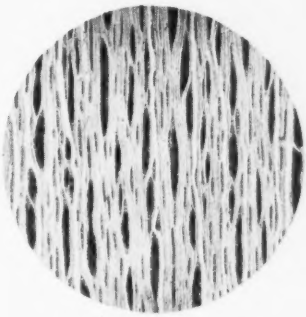

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WETMORE on LENTICELS IN DICOTYLEDONS

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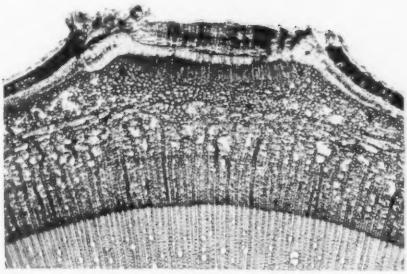

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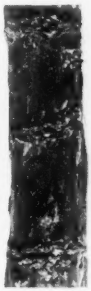

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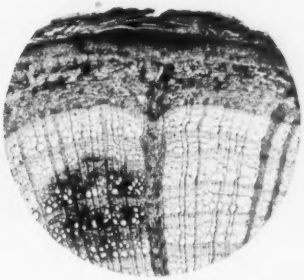

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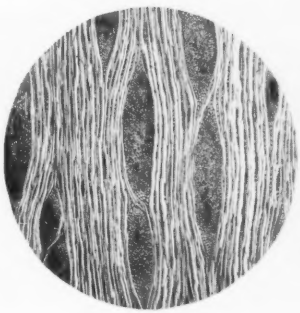

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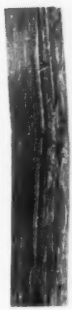

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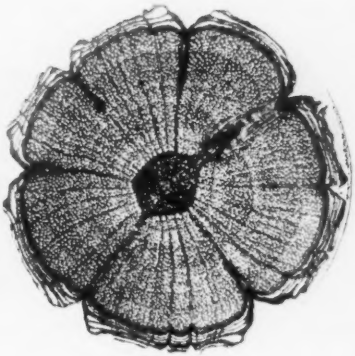

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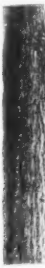
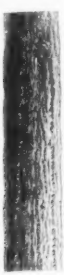
WETMORE on LENTICELS IN DICOTYLEDONS





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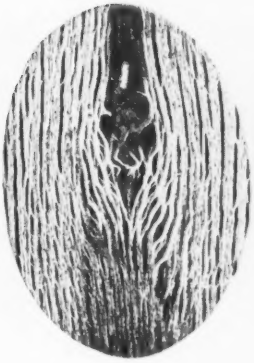

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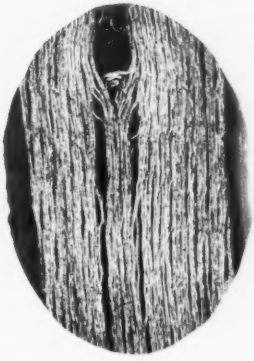

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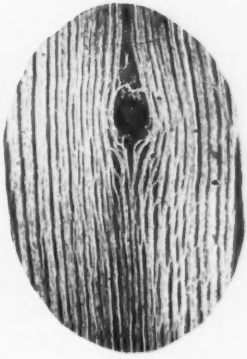

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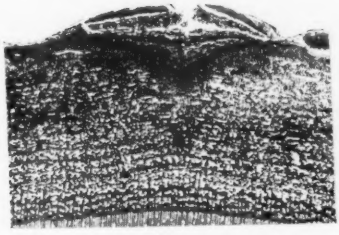

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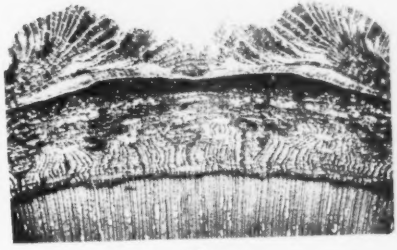

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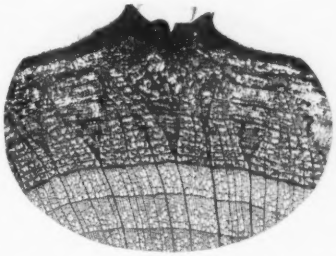

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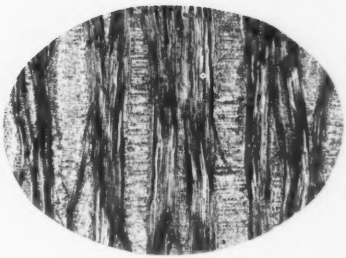

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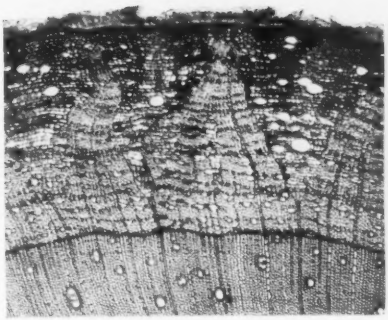

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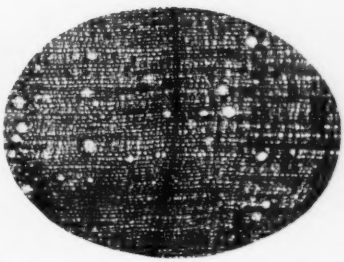

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WETMORE on LENTICELS IN DICOTYLEDONS

FIG. 19.—Segment of stem of *Spiraea salicifolia*, with lenticels in two flanking lines below node, and undivided line above; ×1.5.

FIG. 20.—Segment of branch of *Carpinus cordata*, showing immense longitudinal lenticels; ×1.

FIG. 21.—Segments of stems of *Spiraea gemmata*, with lenticels clustered at nodes; ×1.

FIG. 22.—Tangential section of *Spiraea Veitchii*, showing two flanking lines of multiseriate rays below node; ×10.

FIG. 23.—Same for *S. salicifolia;* ×10.

FIG. 24.—Same for *S. gemmata*, showing multiseriate rays clustered at node; ×10.

FIG. 25.—Portion of transverse section of *Salix alba* var. *vitellina*, with V-shaped rays in phloem, especially under lenticel; ×25.

FIG. 26.—Portion of transverse section of *Carpinus cordata*, showing lenticel and its relation to V-shaped rays of phloem; ×22.

PLATE X

FIG. 27.—Portion of transverse section of *Tilia americana*, showing V-shaped phloem rays and relation of lenticel to one of them; ×25.

FIG. 28.—Tangential section of phloem of *Tilia americana*, showing V̄-shaped rays; ×40.

FIG. 29.—Segment of branch of *Carya ovata*, with vertical lines of longitudinal lenticels; ×1.

FIG. 30.—Portion of transverse section of *Carya ovata*, with prominent V-shaped phloem rays, each subtended by small lenticel; ×25.

FIG. 31.—Segment of stem of *Tilia americana*, with short vertical rows of longitudinal lenticels; ×1.

FIG. 32.—Tangential section of single large V-shaped ray of phloem of *Carya ovata*, showing presence of actual cambium; ×40.

FIG. 33.—Segment of branch of *Amelanchier oligocarpa*, with longitudinal lenticels much elongated vertically; ×1.

INFLUENCE OF MINERAL ELEMENTS UPON DEVELOPMENT OF CHLOROPLAST PIGMENTS OF SOY BEANS[1]

CARL G. DEUBER

(WITH FIVE FIGURES)

Introduction

Cultural experiments with plants have demonstrated many instances in which deficiencies of various mineral elements have produced, in addition to reduced growth, a disturbance in the development of the chloroplast pigments. The commonest manifestation of such a condition is the yellow or white color of the foliage of plants lacking a proper supply of iron. Most of the other essential elements also influence the development of the chloroplast pigments.

VILLE (**24**), working with hemp plants, found that the greatest decrease in chlorophyll and carotin was brought about by a deficiency of nitrates. Lesser decreases resulted from deficiencies of potassium, phosphate, and calcium in the order named. LESAGE and SCHIMPER (**22**) noted that excesses of mineral substances reduced the chlorophyll content of plants. REED and HAAS (**18**) investigated the effects of several ions upon young citrus and walnut trees. They found that chlorosis of the leaves resulted from high applications of chlorine in the form of sodium chloride, from the application of sodium bicarbonate in solutions deficient in calcium, from high concentrations of potassium, and from very low concentrations of magnesium. GARNER and co-workers (**7**) described chloroses of tobacco plants caused by deficiencies of potassium, sulphur, and magnesium. MAMELI (**14**) found that within certain limits the development of the chlorophylls (*a* and *b*) was proportional to the magnesium supplied in the nutrient solution. The mottling of *Coleus* plants was associated with a shortage of nitrates by SCHERTZ (**20**). BOKORNY (**2**) observed that the first effect of cultivating green algae in solutions lacking calcium was a decrease in the chlorophyll content.

[1] Portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Missouri, 1925.

Applications of sulphur-containing fertilizers to sweet corn (**6**), alfalfa (**19**), rutabagas, parsnips, and beets (**3**) resulted in a darker green color of the foliage. McHARGUE (**12**) reported manganese in small amounts essential for proper chlorophyll development. McLEAN and GILBERT (**13**) have corrected a chlorosis of spinach by spraying with manganous sulphate solutions.

While there is abundant evidence that various mineral elements influence the formation of the chloroplast pigments, very little has been accomplished in showing in what manner this influence is operative. A mineral element may function as a constituent of the chlorophylls as magnesium; it may act as a catalyst in the formation of specific portions of the pigment, as is suggested by ODDO and POLACCI (**16**) for iron; or it may not have a direct bearing on the pigments themselves but so influence the general metabolic processes, such as respiration, that the normal production of the chloroplast pigments is more or less impaired.

WILLSTÄTTER (**25**) has shown conclusively that magnesium is the only mineral constituent of chlorophyll (*a* and *b*). The reason for the connection of this element with chlorophyll development demonstrated by MAMELI, GARNER and co-workers, and by REED and HAAS is therefore clear. Nitrogen is also a constituent of chlorophyll. ODDO and POLACCI suggest that iron acts as a catalyst in the formation of the pyrrole groupings which are the basis of the chlorophyll molecules. These investigators arrived at this hypothesis from the successful substitution of a magnesium salt of pyrrole carbonic acid for iron in the growth of plants. Studies by the writer (**4**) with a similar compound substituted for iron with several plants gave negative results in all trials. We do not know in what way the other mineral elements are connected with the formation of the chloroplast pigments.

Not only is our knowledge limited as to the way in which the mineral elements are connected with the development of the chloroplast pigments, but we also know very little of the effect of an element upon the development of the individual pigments found in the chloroplast. Yet in view of present theories of photosynthesis (**1**) which associate all four of the chloroplast pigments with this process such information is important. By comparing the absorption bands

of chlorophylls (*a* and *b*) in living leaves, WLODEK (**27**) has been able to show that a lack of potassium causes an absolute and relative diminution of chlorophyll *b* and an increase in chlorophyll *a*. The data of MAMELI for maize show that increasing the magnesium supply above a certain point results in a decrease in the quantity of carotinoids, although the chlorophylls were further increased.

The work reported in this paper and elsewhere by the writer was planned to determine the effect of several essential mineral elements on the development of the chloroplast pigments, and to secure information on the method of their action. In the present investigation soy bean plants were grown in water cultures, and the influence of iron, potassium, and sulphur in the development of the chloroplast pigments studied by making quantitative determinations of the chlorophylls and the carotinoids.

Methods

The culture vessels employed were 500 cc. wide-mouth bottles wrapped in black paper and provided with paraffined brown paper tops. Three or four Wilson soy bean seedlings were grown in each culture. Iron wire rings with uprights were fastened to the culture vessels with rubber bands to support the tops of the plants. In the experiments with iron salts these supports were new, so that iron from iron rust was not a factor.

Knop's nutrient solution with the following composition was used: $Ca(NO_3)_2$, 0.8 gm.; KNO_3, 0.2 gm.; KH_2PO_4, 0.2 gm.; $MgSO_4$, 0.2 gm.; distilled water, 1000 cc. Separate stock solutions of the constituent salts were prepared 100 times the concentration used in the cultures, dilution and mixing of the salts being made just before renewing the culture solutions every four or five days. Stock solutions of ferric citrate and ferrous sulphate were prepared fresh at each renewal of the solutions, to prevent hydrolysis of the iron salts. Merck's "reagent" and Baker's "analysed" chemicals were used, and the distilled water was obtained from a tripure still.

In the experiments with potassium, the basal Knop's solution was modified to contain 0, 28, 134, and 238 p.p.m. K. This element was omitted from the first solution by substituting NaH_2PO_4 for KNO_3 and KH_2PO_4. The second solution contained 28 p.p.m. K in

the form of KH_2PO_4, and the third with 134 p.p.m. K was the regular Knop's solution. The fourth solution contained 238 p.p.m. K in the forms of KNO_3, KH_2PO_4, and KCl.

In the experiment with sulphur, the basal Knop's solution was modified to contain 0, 13, 26, and 46 p.p.m. S. Sulphur was omitted from the first solution by replacing $MgSO_4$ with $MgCl_2$. The second solution with 13 p.p.m. S received but half the $MgSO_4$ of the Knop's solution, the Mg content being maintained by adding $MgCl_2$. Knop's solution contains 26 p.p.m. S, the concentration employed in the third solution. The fourth solution with 46 p.p.m. S received Na_2SO_4 in addition to the $MgSO_4$ of the Knop's solution. Iron was supplied to the solutions of the potassium and sulphur experiments in the form of ferric citrate at the rate of 0.228 p.p.m. iron.

The reactions of the nutrient solutions were determined at the time of preparing the solutions and after the plants had grown in them four or five days, the colorimetric method of GILLESPIE (**8**) being employed.

The experiments were conducted on central benches in a well lighted greenhouse having an average temperature of 20° C.

In the first experiments with iron and potassium, the chloroplast pigments were determined by extracting the leaves of the plants which had been air dried in the dark at approximately 18° C. In the later experiments the chloroplast pigments were extracted and estimated immediately upon harvesting the fresh plants. The methods of WILLSTÄTTER, modified as suggested by SCHERTZ[2] for the extraction and separation of the chloroplast pigments were followed. The procedure consisted in extracting the leaf material with acetone, transferring the acetone extract to ether, and saponifying the chlorophylls in order to separate them from the carotinoids. Carotin and xanthophyll were separated by means of their different solubilities in petroleum ether and methyl alcohol.

The acetone extracts of a series of leaf samples were compared relatively in a Dubosque colorimeter. The ether extracts containing

[2] The writer wishes to acknowledge his thanks to Dr. F. M. SCHERTZ, United States Bureau of Plant Industry, for suggestions regarding the extraction, separation, and quantitative estimation of the chloroplast pigments, and for a sample of purified chlorophyll (*a* and *b*) mixed.

all the chloroplast pigments were treated in the same way. The mixed chlorophylls (*a* and *b*) of a given series of samples were compared relatively and with alcoholic solutions of chlorophylls (*a* and *b*), the purified chlorophyll for these standards being furnished by SCHERTZ. Relative comparisons were made of the combined carotinoid samples and of the carotin and xanthophyll.

Influence of iron

Two experiments were performed on the influence of iron as ferrous sulphate and as ferric citrate, on the growth and on the chloroplast pigments of soy bean plants. In the first experiment Knop's solution containing iron as ferrous sulphate at rates of 0.367, 0.734, 1.468, 2.936, 4.411, 5.872, and 8.822 p.p.m., and iron as ferric citrate at rates of 0.228, 0.456, 0.912, 1.824, 2.736, 3.648, and 5.472 p.p.m. Two cultures of three plants each were used for each concentration of iron. The solutions were renewed every four days. The total quantity of iron supplied per plant in milligrams was 1.5 times the concentration in parts per million. The initial hydrogen-ion concentration varied from P_H 5.65 to 5.8, and the maximum final from P_H 6.15 to 6.7, the greater changes occurring with the larger plants.

After twelve days' growth, chlorosis of the new leaves in both series of iron salts was evident with the first three concentrations of iron and the solution lacking iron. The chlorotic condition was soon followed by a black spotting of the youngest leaves. This condition first appeared in the form of minute yellow areas in the leaf blades; these increased to irregular shaped areas less than 1 mm. in size, and underwent changes in color to light red, red, and finally to reddish brown or black. Young leaves were often peppered over their entire surface with these spots and dropped off prematurely. This condition was most evident in the ferrous sulphate series. At the conclusion of the experiment, after 35 days' growth, chlorosis was evident in the first five solutions of the ferrous sulphate series and in the first four of the ferric citrate series.

The growth of the plants was markedly affected by the concentration or amount of iron supplied.[3] Increasing the concentration of

[3] It is impossible to differentiate in these experiments between concentration and total amount of an element supplied. In a given series the nutrient solutions were

iron as ferric citrate produced improved growth up to 3.648 p.p.m. (fig. 1). Increasing iron as ferrous sulphate increased growth up to 8.822 p.p.m., the maximum used.

Ferric citrate proved to be a much more efficient source of iron than ferrous sulphate at all concentrations employed. A comparison of the growth curves of fig. 1 makes it possible to estimate the

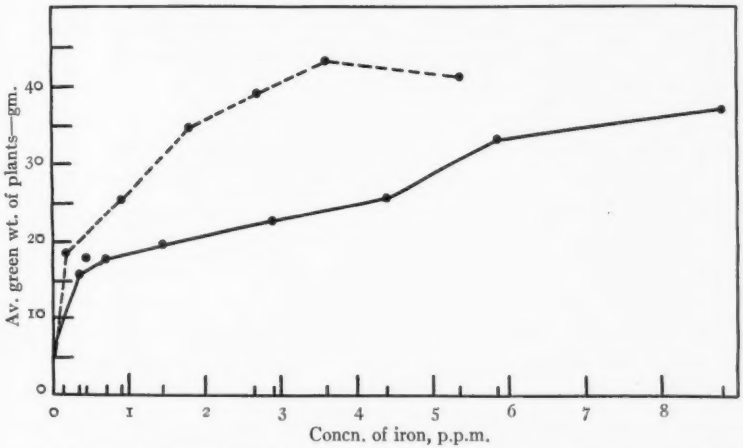

FIG. 1.—Green weights of soy bean plants per culture supplied seven concentrations of iron as ferrous sulphate and ferric citrate (p.p.m. = parts per million). Dotted line, ferric citrate; continuous line, ferrous sulphate.

quantity of iron as ferric citrate which would be required to produce the same amount of growth obtained with the various concentrations of ferrous sulphate iron. Thus to produce 37.155 gm. fresh weight of plants secured with 8.822 p.p.m. iron as ferrous sulphate would require 2.3 p.p.m. ferric citrate iron, or 3.8 times as much iron as ferrous sulphate as in the form of ferric citrate. For a concentration of 5.872 p.p.m. iron as ferrous sulphate this factor is 3.5; for a concentration of 4.411 p.p.m., 4.4; for a concentration of 2.936 p.p.m., 4.5; and for a concentration of 1.468 p.p.m. it is 4.9. In other words,

changed the same number of times. The total quantity of an element supplied varied with the concentration. When the term concentration is used in this paper it is understood that total quantity or amount of the element supplied may be the governing factor and not the concentration.

iron as ferric citrate in the range of concentrations where marked depression of growth did not occur was about four times as efficient in this experiment as iron in the form of ferrous sulphate. Since the highest concentration of ferrous sulphate employed produced no decrease in growth its optimum concentration cannot be stated, but for ferric citrate a concentration of 3.648 p.p.m. iron was the optimum.

Attempts to determine the plastid pigments were made by extracting with acetone two gm. samples of leaves dried as previously described. When the green pigments of the acetone extracts were transferred to ether, some of the greenest leaf samples gave a very green wash water, which would not give up its coloring matter to ether, petroleum ether, or carbon bisulphide. More pigment was transferable to ether from the acetone extracts of some of the chlorotic leaf samples than from that of some of the greenest and most vigorous plants. Evidently, during the drying of the leaves some of the chloroplast pigments were decomposed to a water soluble form. Comparisons of the total pigment material extracted from dried leaves by 80 per cent acetone appeared unreliable. In the ferric citrate series the highest pigment content was found in the plants grown in a solution containing 0.456 p.p.m. iron. These plants had yellow to light green leaves. From these results it was concluded that acetone extracts of dried soy bean leaves are unsafe criteria for the estimation of the chloroplast pigments, and that satisfactory quantitative determinations of the chloroplast pigments may be seriously interfered with by changes in the pigments occurring during the drying of the leaves. Although WILLSTÄTTER (11) used the dried powder of leaves for all ordinary extractions, he found that drying caused a loss of chlorophyll, and when the leaves were not properly dried the pigments were altered. SCHERTZ strongly advises using fresh leaves for all quantitative pigment extractions.

In the second experiment soy bean plants were grown thirty days (January 18–February 17, 1925) in Knop's solution supplied iron as ferrous sulphate at rates from 0.367 to 7.340 p.p.m. iron, as indicated in table I, and in the form of ferric citrate at rates from 0.228 to 4.560 p.p.m. as given in table II. Four cultures of four plants each were employed for each concentration of iron. The solutions were

renewed every five days. The total quantity of iron supplied in mg. per plant was 0.83 times the concentration in p.p.m. The initial hydrogen-ion concentration of the solutions varied from P_H 5.5 to 5.7.

The foliage of the plants in solutions 1 and 2 of both series became chlorotic. The black spot condition of the leaves was most pronounced in solutions 1, 2, and 3 of the ferrous sulphate series. Immediately upon determining the green weights of the plants, the pigments of 10 gm. samples of the fresh leaves were extracted with acetone.

TABLE I

AVERAGE GREEN WEIGHTS OF SOY BEAN PLANTS PER CULTURE AND RELATIVE VALUES OF CHLOROPLAST PIGMENTS OF 10 GM. FRESH LEAVES, FERROUS SULPHATE SERIES

	SOLUTION AND LEAF SAMPLE NUMBER				
	1	2	3	4	5
Concentration of iron, p.p.m.	0.000	0.367	1.835	3.670	7.340
Green weight in gm.	7.705	10.423	13.158	13.651	14.925
Ether extract of chlorophyll and carotinoids	0.78	1.00	1.00	1.02	0.95
Chlorophyll (*a* and *b*)*	0.0045	0.0104	0.0134	0.0137	0.0147
Total carotinoids	0.55	1.00	0.64	1.00	0.74
Carotin	0.55	1.00	0.97	1.42	1.48
Xanthophyll	0.52	1.00	0.87	1.18	0.97

* Grams of purified chlorophyll (*a* and *b*) equivalent to the chlorophyll (*a* and *b*) from 10 gm. of fresh leaves.

The chloroplast pigments were transferred without loss from acetone to ether, and the relative values of the combined chlorophylls and carotinoids determined by comparison with those of sample 2 of each series. The chlorophyllin salts were secured and compared with an alcoholic solution of 0.257 gm. of purified chlorophyll (*a* and *b*) in 250 cc. The combined carotinoids, carotins, and xanthophylls were then compared relatively with those of sample 2. The values for these determinations are summarized in tables I and II.

Ferric citrate was again the most efficient source of iron, but in this experiment the order of efficiency was much higher than in the previous one. For solution 5 the iron of ferric citrate was 13.3 times as efficient as that of ferrous sulphate, for solution 4, 14.7, and for

solution 3, 12.2. The optimum concentration of iron as ferric citrate was 2.280 p.p.m., but with ferrous sulphate the highest concentration employed (7.340 p.p.m.) gave the most growth.

With both ferrous sulphate and ferric citrate an increase in the iron supply to the plants resulted in increases of the chlorophylls of a unit of fresh leaf material. An exact proportional relation did not exist between the iron supplied and the amount of chlorophyll found per unit of leaf material. A very good correlation appears to exist between the chlorophyll content per unit of fresh leaves and growth,

TABLE II

AVERAGE GREEN WEIGHTS OF SOY BEAN PLANTS PER CULTURE AND RELATIVE VALUES OF CHLOROPLAST PIGMENTS OF 10 GM. FRESH LEAVES, FERRIC CITRATE SERIES

	Solution and leaf sample number				
	1	2	3	4	5
Concentration of iron, p.p.m.	0.000	0.228	1.110	2.280	4.560
Green weight in gm.	7.984	13.968	16.988	18.732	17.928
Ether extract of chlorophyll and carotinoids	0.38	1.00	1.26	1.53	1.60
Chlorophyll (*a* and *b*)*	0.0058	0.0087	0.0138	0.0160	0.0160
Total carotinoids	0.46	1.00	1.00	1.07	1.47
Carotin	0.22	1.00	1.11	1.21	1.53
Xanthophyll	0.34	1.00	0.96	1.21	1.49

* Grams of purified chlorophyll (*a* and *b*) equivalent to the chlorophyll (*a* and *b*) from 10 gm. of fresh leaves.

as can readily be seen from the curves of fig. 2. The values of the chlorophylls (*a* and *b*) in terms of purified chlorophyll (*a* and *b*) almost equal the growth of the plants when multiplied by 1000, except with the samples from plants grown without iron.

The values of the total chloroplast pigments in the ether extracts and the carotinoids present some relations that differ from the chlorophyll values. In the ferrous sulphate series the total chloroplast pigments from leaves of plants grown in solution 1 show a marked diminution, but those of solutions 2, 3, 4, and 5 give practically the same values. The leaves of the plants in solution 2 were light green, while those in solutions 3, 4, and 5 were normal green. Why this difference was not evident in the ether extracts cannot be explained. In the ferric citrate series the pigments in the ether ex-

tracts increased in their relative values from solution 1 to 5, which would appear logical judging from the values of the chlorophylls. The combined carotinoids of samples 2 and 4 of the ferrous sulphate series, and samples 3, 4, and 5 of the ferric citrate series exhibited a greenish cast which could not be removed by washing with distilled water, 1 per cent sodium carbonate solution, or by filtering through

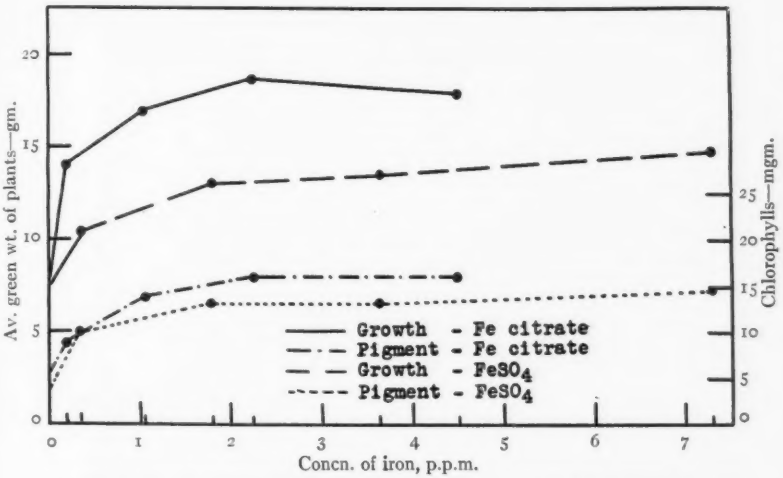

FIG. 2.—Green weights of soy bean plants per culture supplied four concentrations of iron as ferrous sulphate and ferric citrate, and amounts of chlorophyll (*a* and *b*) produced per 10 gm. fresh leaves; chlorophyll (*a* and *b*) expressed in terms of purified chlorophyll (*a* and *b*).

anhydrous sodium sulphate. This made it difficult to make accurate relative comparisons.[4] The separated carotins and xanthophylls of both series exhibited increases with increased iron supply except in the case of sample 5, ferrous sulphate series, where a reduction was found.

Influence of potassium

Two experiments were performed on the influence of potassium on the growth, and on development of the chloroplast pigments of soy bean plants. In the first experiment the plants were grown

[4] SCHERTZ suggested that the greenish cast of the carotinoid solutions might result from the incomplete saponification of the ether extracts.

thirty-six days (October 12–November 17, 1924) in Knop's solution lacking potassium and containing 28, 134, and 238 p.p.m. potassium. Solution 3 with a concentration of 134 p.p.m. potassium, the normal Knop's solution, was considered the control solution. Each solution was replicated with four cultures of four plants each. The solutions were changed every four days.

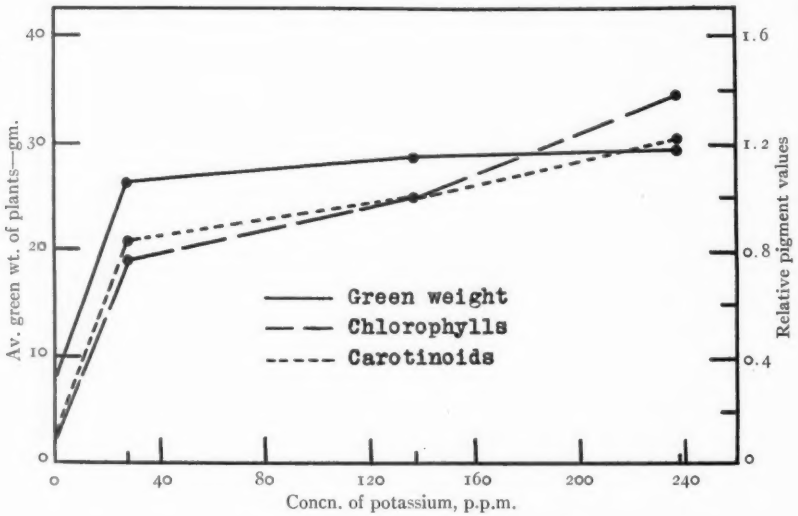

FIG. 3.—Green weights of soy bean plants per culture supplied three concentrations of potassium, and relative values of chlorophyll (*a* and *b*) and carotinoids of 2 gm. samples of dried leaves.

The growth of the plants in the solution lacking potassium was very much stunted, and the leaves developed large translucent areas on the blades and dark red areas along the stems. The plants in the solutions containing potassium made rapid growth. Only small increases in green weight were secured with additions of potassium above 28 p.p.m. (fig. 3). No differences in color of the leaves were observable between the plants in the low and high potassium-containing solutions.

The tops of the plants were air dried in the dark and the pigments extracted from 2 gm. samples of the leaves. In sample 1 some

difficulty was experienced in transferring all the green pigment to ether from the acetone extract. The relative values of the four chloroplast pigments, the chlorophylls, and the carotinoids in ether were determined by comparison with those of sample 3, which were given a value of 1.0. The chlorophyllins were also compared with a solution of 0.0025 gm. of purified chlorophyll (*a* and *b*) in

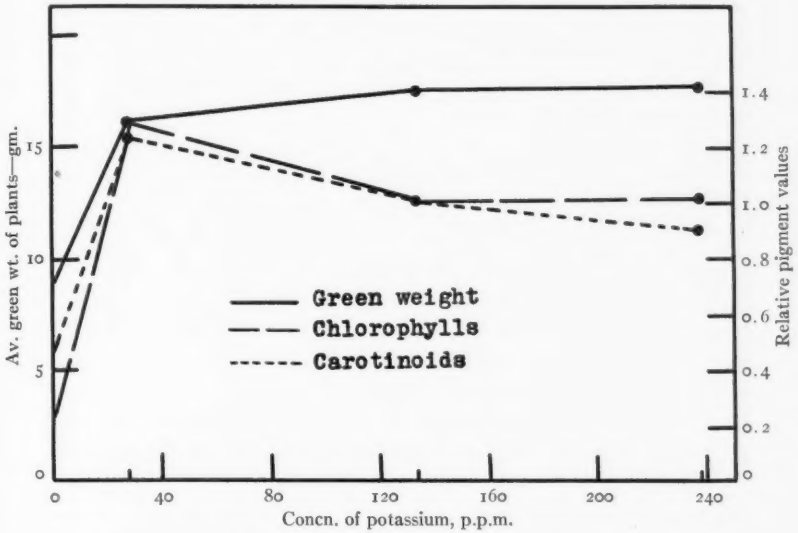

FIG. 4.—Green weights of soy bean plants per culture supplied three concentrations of potassium, and relative values of chlorophyll (*a* and *b*) and carotinoids of 10 gm. samples of fresh leaves.

100 cc. of alcohol. The results of these determinations are shown in fig. 3.

The chloroplast pigments of the leaves were greatly depressed when potassium was withheld from the plants, but the addition of 28 p.p.m. potassium to the nutrient solution resulted in a very large increase in these pigments. Rates of 134 and 238 p.p.m. potassium produced further increases in all the pigments except the xanthophyll of sample 4, which was lower than that of sample 3.

The extraction of the chloroplast pigments from the dried leaf samples in this case was apparently satisfactory. Observable loss of

the chloroplast pigments occurred only in sample 1. This was in distinct contrast to the results secured with the dried leaves of the series with iron. The difference can probably be accounted for by differences in the rapidity of drying of the two lots of leaves, although both were dried in the dark at room temperatures. Although no loss of pigment, with the exception noted, was observed in the wash water from the acetone extracts, the chlorophyll found in the dried leaves was much less than found later in fresh leaves. Expressing the chlorophylls in terms of purified chlorophyll (*a* and *b*), the maximum found in this series in 2 gm. of dried leaves was 5.3 mg., and in the second series with potassium in 10 gm. of fresh leaves the maximum was 18.0 mg. This would suggest a considerable loss of pigments during the drying process, assuming that the leaves contained approximately 80 per cent moisture.

The second experiment with potassium was a replicate of the one already described, and was performed to secure fresh leaves for the extraction of the pigments. The cultures contained four plants each. The solutions were changed every five days during a growing period of thirty days (January 14–February 13, 1925).

At the end of the experiment the plants in the solution lacking potassium were stunted, the leaves crinkled with large white areas in the blades, while some of the older leaves were turning yellow. Many of the stems exhibited dark red to brown areas. The new leaves of the plants in the solution containing 28 p.p.m. potassium were yellowish green and crinkled. The solutions with the highest concentrations of potassium, 134 and 238 p.p.m., produced plants that were normal in every respect.

The pigments of 10 gm. samples of the fresh leaves were extracted immediately upon harvesting the plants. The ether extracts containing the chlorophyll (*a* and *b*) and carotinoids, and the combined carotin and xanthophyll were compared relative to those of sample 3. The chlorophylls were compared with a solution of 0.0257 gm. purified chlorophyll in 250 cc. of alcohol. A summary of these determinations is given in table III.

The chloroplast pigments were markedly reduced when potassium was omitted from the nutrient solution, but showed a very great increase with a concentration of 28 p.p.m. of this element. The

values obtained for sample 2 appear to be too high when compared with those of samples 3 and 4, because at the time of harvesting the plants many of the leaves in solution 2 were slightly chlorotic, while those in solutions 3 and 4 were normally green. The series in which dried leaves were used for extraction also shows no such high chlorophyll content for the plants in solution 2. Duplicate samples of the fresh leaves in the second series gave similar high results for sample 2. The chlorophylls of sample 4 when compared with a solution of purified chlorophyll (*a* and *b*) were greater than those of sample 3, but relative comparisons of these pigments gave approximately the

TABLE III

AVERAGE GREEN WEIGHTS OF SOY BEAN PLANTS PER CULTURE AND RELATIVE VALUES OF CHLOROPLAST PIGMENTS OF 10 GM. FRESH LEAVES, SECOND POTASSIUM SERIES

	SOLUTION AND LEAF SAMPLE NUMBER			
	1	2	3	4
Concentration of K, p.p.m.	0.000	28	134	238
Green weight in gm.	8.995	16.178	17.722	17.756
Ether extract of chlorophyll and carotinoids	.....	1.20	1.00	0.95
Chlorophyll (*a* and *b*)	0.22	1.32	1.00	1.07
Chlorophyll (*a* and *b*)*	0.0030	0.0180	0.0136	0.0146
Total carotinoids	0.48	1.25	1.00	0.90

* Grams of purified chlorophyll (*a* and *b*) equivalent to the chlorophyll (*a* and *b*) from 10 gm. of fresh leaves.

same values. The carotinoids of sample 4 showed a slight decrease as compared with those of sample 3.

Influence of sulphur

Soy bean plants were grown in solutions lacking sulphur and containing 13, 26, and 46 p.p.m. of this element. Four plants were placed in each culture and the cultures replicated four times for each solution. The solutions were renewed every five days during a growing period of forty-three days (January 19–March 3, 1925).

A slight chlorosis of the new leaves of the plants in the solution containing the highest concentration of sulphur (46 p.p.m.) occurred in the fourth week, but by the end of the growing period these leaves were normal green. Some of the leaves were crinkled and developed

white to rust colored areas in the blades, together with a drying of the leaf margins. A similar crinkling of new leaves and discolored areas in the blades were also found in the plants deprived of sulphur. A swelling of lateral roots was noted in the plants lacking a supply of sulphur and those receiving the lowest concentration of this element.

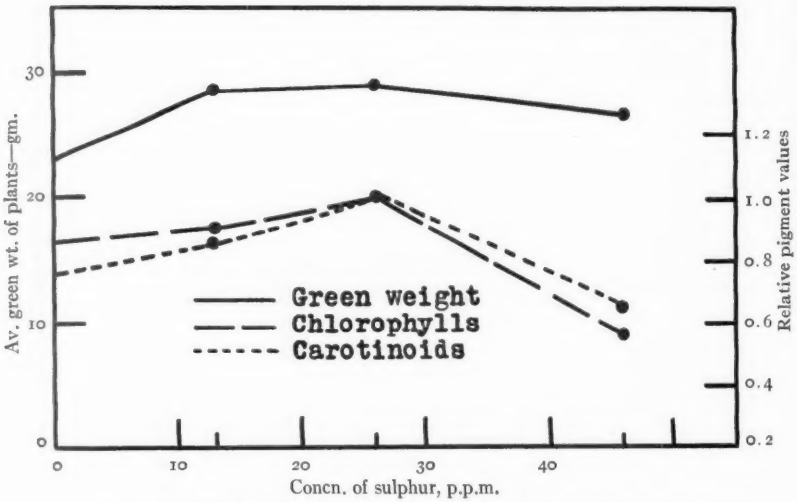

FIG. 5.—Green weights of soy bean plants per culture supplied three concentrations of sulphur, and relative values of chlorophyll (*a* and *b*) and carotinoids of 10 gm. samples of fresh leaves.

The growth of the plants was only slightly influenced by the concentration or amount of sulphur supplied in the nutrient solutions, as indicated by the green weight data of table IV. DUGGAR (**5**) states that sulphur is usually required in such limited quantity that the seeds may furnish all that is needed for normal growth of plants through a considerable period. HART and PETERSON'S (**9**) data on the sulphur content of a number of seeds show that soy bean seeds are high in this element. Soy beans contain 0.341 per cent sulphur, wheat 0.170, corn 0.164, and oats 0.189 per cent.

The chloroplast pigments of 10 gm. samples of fresh leaves were extracted and determined relatively. These data are given in table

IV. The maximum development of the chloroplast pigments per unit of fresh leaves was found in the plants grown in the regular Knop's solution (solution 3) with sulphur at a concentration of 26 p.p.m. A decrease or an increase in the sulphur supplied as compared with that of Knop's solution decreased the quantity of chloroplast pigments, the excess sulphur supply causing the greater depression.

TABLE IV

AVERAGE GREEN WEIGHTS OF SOY BEAN PLANTS PER CULTURE AND RELATIVE VALUES OF CHLOROPLAST PIGMENTS OF 10 GM. FRESH LEAVES, SULPHUR SERIES

	SOLUTION AND LEAF SAMPLE NUMBER			
	1	2	3	4
Concentration of S, p.p.m.	0.000	13	26	46
Green weight in gm.	23.340	28.517	28.959	26.949
Ether extract of chlorophyll and carotinoids	0.71	0.83	1.00	0.58
Chlorophyll (*a* and *b*)	0.86	0.90	1.00	0.57
Chlorophyll (*a* and *b*)*	0.0092	0.0094	0.0107	0.0058
Total carotinoids	0.76	0.86	1.00	0.65
Carotin	1.03	0.81	1.00	0.46
Xanthophyll	0.98	0.80	1.00	0.46

* Grams of purified chlorophyll (*a* and *b*) equivalent to the chlorophyll (*a* and *b*) from 10 gm. of fresh leaves.

Discussion

Of the three mineral elements studied, the lack of iron was found to limit the growth of soy bean plants most, followed by potassium. Sulphur had a relatively slight influence on the growth of these plants. The source of the iron presented some interesting features in connection with growth. Ferric citrate in the first experiment proved to be about four times as efficient a source of iron as ferrous sulphate, and in the second experiment about thirteen times as efficient. The difference in the value of this factor for the two experiments is probably due to differences in environment, and to the fact that one series was grown for five weeks and the other for four weeks. The relative constancy of this factor of efficiency in each of these experiments indicates a constant difference in the availability of iron in the citrate and sulphate. The cause of the difference in efficiency of the iron in these two salts may be due to a lower solubility of the

ferrous sulphate, the ferric or ferrous state of the iron or the anion with which the iron is combined. Of these possible causes, that of solubility appears to be the most probable. TOTTINGHAM and RANKIN (**23**) found that the solubility of iron as ferric citrate was about four times that of iron as ferrous sulphate in the Livingston-Tottingham solution R_8C_1 at a P_H of 6.0. A similar constant relation can also be found in the data of JONES and SHIVE (**10**) with ferric phosphate and ferrous sulphate in the growth of wheat. Ferrous sulphate was approximately 1.6 times more efficient than the less soluble ferric phosphate.

The optimum concentrations of ferric citrate were 2.280 and 3.648 p.p.m. iron, but the highest concentrations of ferrous sulphate employed (7.340 and 8.822 p.p.m. iron) produced no decrease in growth, so the optimum concentration was not attained. MARSH and SHIVE (**15**) obtained the highest yield of soy bean plants with ferrous sulphate at the rate of 19.4 mg. iron per liter for three plants, or 6.4 mg. per plant over a growing period of five weeks when the iron supply was adjusted at frequent intervals. In the present experiments a supply of 13.233 and 6.116 mg. iron as ferrous sulphate per plant, over growing periods of five and four weeks respectively, gave very satisfactory growth.

The value of the determination of the chloroplast pigments depends upon the reliability of the methods used for such determinations. The operation is tedious and complicated, and there are numerous opportunities for error. It was found that the pigments of fresh leaves could be extracted much more easily and completely than those from dried ones. The acetone extracts from dried leaves often contained water-soluble green pigment which could not be transferred to ether. Determinations of the separated chlorophyllins from such extracts gave values that were obviously too low, which indicated a decomposition of the pigments during drying. Relative comparisons of 80 per cent acetone extracts of the total pigment content of leaf samples were not satisfactory, because of the presence of water soluble pigments. A comparison of the total chloroplast pigments by transferring from acetone to ether and washing out all water soluble material has the double disadvantage of permitting relative determinations only and of including both yellow and green

pigments. The comparison of the chlorophylls with known solutions of chlorophyll (*a* and *b*) gave the most satisfactory results, because the colors were comparable, and because, while not expressing exactly the quantity of chlorophyll present, it did allow a comparison of the amount of these pigments among different experiments. The carotinoids combined and separated were particularly difficult to determine accurately. A greenish tint impossible to remove in some of the carotinoid extracts made relative comparisons difficult. The cause of this variation in color could not be determined. The addition of concentrated sulphuric acid to a carotinoid extract in petroleum ether produces a clear green color, the original yellow color returning upon dilution with water. It is possible that a partial change of the carotinoids to this greenish form may occur during some stage in the manipulation. SCHERTZ is of the opinion that the greenish tint is the result of incomplete saponification of chlorophyll (*a* and *b*) altered probably by drying. Nevertheless, certain facts of interest appear evident from the determinations of the chloroplast pigments. The omission of iron or potassium from the solutions reduced the amount of the chloroplast pigments decidedly more than the omission of sulphur. This, however, does not mean that if sulphur were seriously deficient the pigment production would not be affected. The relatively high sulphur content of soy bean seeds probably supplies the seedlings with enough for a considerable period. The general tendency was for the pigments to increase with increasing supplies of iron and sulphur, but with the highest concentration of sulphur (46 p.p.m.) a very decided decrease in pigment content resulted. Judging from the results secured from the fresh leaves, the higher concentrations of potassium also caused a decrease in the amount of the chloroplast pigments.

One rather unexpected feature of the analyses is the fact that the carotinoids and chlorophylls vary for the most part together. A reduction in the chlorophylls is associated with a similar reduction in the carotinoids. This suggests that the formative processes involved in the production of these two types of pigments are influenced similarly by the essential elements included in this study. It also suggests that the yellowish colors frequently observable in chlorotic leaves are not due to carotin or xanthophyll but to xan-

thones, flavones, or related yellowish pigments. There is no evidence from the data to indicate that the formation of the chlorophylls is more dependent upon iron, potassium, or sulphur than the formation of the carotinoids. On the contrary, the determinations emphasize the close relationship which appears to exist between these two groups of chemically different pigments.

Summary

1. Soy bean plants were grown in nutrient solutions in which the concentrations of the iron, potassium, and sulphur contents were varied.

2. Ferric citrate was found to be four to thirteen times more efficient an iron source than ferrous sulphate. This difference in efficiency was constant in a given experiment.

3. Small differences in the iron content of the nutrient solutions produced considerable differences in the growth of the plants.

4. Iron deficiency caused small black spots to develop in the chlorotic new leaves.

5. Lack of potassium stunted growth markedly, but concentrations of 28–238 p.p.m. of this element produced about the same amount of growth.

6. The sulphur content of the nutrient solution influenced growth only slightly.

7. Acetone extracts of the total pigments of leaves are not safe criteria for judging the relative chloroplast pigment development.

8. Ether extracts of the chloroplast pigments freed from water soluble pigments are more satisfactory for judging the relative chloroplast pigment content than acetone extracts.

9. The separated carotinoid pigments may show greenish tinges which interfere with their quantitative estimation.

10. Estimation colorimetrically of chlorophyll (*a* and *b*) against a standard of purified chlorophyll (*a* and *b*) in alcohol was found to be a very satisfactory method.

11. The lack of iron or potassium in the nutrient solution resulted in a more marked depression of the chloroplast pigments than a lack of sulphur.

12. In general, the chloroplast pigments increased with the in-

creasing concentrations of iron and sulphur. The highest concentration of sulphur used (46 p.p.m.) caused a marked decrease in the pigment content of the leaves.

13. Concentrations of potassium above 28 p.p.m. reduced the chloroplast pigments when the extractions were made from fresh leaves.

14. In all the experiments the chlorophylls (*a* and *b*) and carotinoids were influenced by the composition of the nutrient solution to about the same extent.

15. An exact proportional relation could not be established between the concentration or amount of any of the elements studied and the amount of pigment formed.

16. A correlation between the green weight of the plants and the chlorophyll (*a* and *b*) existed in one experiment with iron, but did not apply in the other experiments.

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TRANSLOCATION OF FATS AS SUCH IN GERMINATING FATTY SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 351

J. B. RHINE

Introduction

Among the problems that challenge our interest in connection with the translocation of foods, the one which has proved most baffling to the investigator is that of explaining the forces responsible for the movement of solutes from cell to cell through long distances in the plant. No generally satisfactory explanation has yet been proposed, although several have been suggested. This is true of the soluble compounds, but it would be much more difficult to explain the movement from cell to cell of insoluble bodies, granules, or droplets, of which the fats are an example. Indeed the propulsion of fat droplets through the plant, if this occurs, is a striking question in plant dynamics.

The statement that fats can move as such from cell to cell is frequently found in the literature, and usually specifies that they probably move in the form of "fine emulsions." A canvass of a limited circle of plant physiologists revealed the fact that there existed great uncertainty on this point, some thinking the fats moved as fats in the emulsified state, others supposing that they moved as fatty acids and glycerol, and still others believing there was not enough evidence on the subject to justify a statement. It appears that no actual work on this problem has been reported in the last thirty-five years. The older work is cited in support of fat movement in such standard texts as those of PFEFFER, JOST, and BENECKE-JOST, and those on plant chemistry by CZAPEK and by HAAS and HILL.

SACHS (4) in 1859 observed the presence of considerable quantities of fat in the hypocotyls of germinating fatty seeds, and was led to the conclusion that it had moved out from the seed in that form. His *Physiology of plants* of 1889 contains the following statement of

his position: "It thus appears that the fats can pass the closed tissue-cells as such; though of course the greater part of them is transformed to starch and sugar for transport and use." SCHMIDT (5) cites PETER'S work as supporting SACHS' claim by quantitative determination of the fat in the hypocotyl. PETER, too, thought the fats moved as fats. SCHMIDT, working in PFEFFER'S laboratory in 1890, arrived at the same conclusion from similar observations.

SCHMIDT, however, went much further in his extensive experiments. He planned elaborate tests to see whether the living cell could take up oils from the outside. First he tried the fungi, and, although he was apparently successful, he did not consider the results conclusive, or applicable to the higher plants. Next the mosses were used, but here it was found necessary to dry the moss leaves for eight days at 28° C., and then use a partial vacuum in order to get the oils to penetrate the walls. This was not analogous to the situation obtaining in the tissues in which he thought the oils moved, and he did not consider it as evidence. Finally, SCHMIDT tried the injection of oils into etiolated starved pea seedlings, thinking that in this case the intake of oil by the cells would be evidence of the possibility of fat movement from cell to cell in the normal plant. His method consisted in growing pea seedlings in the dark until the food was exhausted from the cotyledons (parts of which were cut away to hasten starvation). A longitudinal incision was then made just above the ground, and a strip of filter paper, saturated with the oil to be used, was inserted in the incision. If colored oils were used the rise and descent of the oil through the intercellular spaces could be observed. By a very difficult technique and by using longitudinal sections, the presence of oil drops within the cells could be determined with a moderate degree of certainty. His results may be stated briefly as follows: Using almond oil and oleic acid, he found that the more acid there was mixed with the oil, the more rapid the rise. With pure fatty acid the cell was penetrated and droplets were found within the cytoplasm after some hours' standing. SCHMIDT reports that the neutral oil did not enter the cell unless there was at least 10 per cent free acid present. This led him to propose an explanation which involved the formation of soaplike compounds with the free acid and the emulsification of the neutral fat by these soapy

substances. He thought it passed through the wall as a "fine emulsion," and assumed from this that the fats could pass from cell to cell.

SCHMIDT also studied germinating fatty seeds. His most important evidence for fat movement from these studies is the fact that he found no sugar or starch in the endosperms during germination. He assumed from this that the fats do move as fat from the cells of the endosperm to the cotyledons.

This is the case for fat movement, so far as could be ascertained from the literature. No evidence was reported against it. The essential points of SCHMIDT'S work have been repeated, and then further experimentation was undertaken with germinating fatty seeds, in the hope of finding a satisfactory basis for the solution of the problem. The results of both phases of the work are reported here.

Investigation

The first point to be investigated was the reported intake of fats by the cells of etiolated pea seedlings. Seedlings were grown until they were in a starved condition, incised longitudinally, and a strip of filter paper saturated with oil colored with Sudan III was then inserted. The oils used were linseed, linseed fatty acids, olive oil, oleic acid, and castor oil, giving a wide range of viscosity, as may be seen in table I, taken from LEWKOWITSCH (**2**).

TABLE I

VISCOSITY OF CERTAIN OILS AT 15° C. (SCHÜBLER)

OIL	VISCOSITY
Castor oil	203.3
Olive oil	21.6
Almond oil	16.6
Linseed oil	9.7
Distilled water	1.0

Almond oil was not used, but its place in the viscosity range may be seen in the table. Following SCHMIDT'S procedure, the seedlings were tested with the various oils, and, using the dissolved stain as an indicator, the rate of rise of each in the stem was observed. Table II gives the results.

The movement of the oils was through the intercellular spaces, and was confined mainly to the outer regions of the cortex. In some cases the oil would get into the tracheae and seemed to be drawn up by the pull of transpiration. Such cases were disregarded.

TABLE II

RISE OF OILS IN PEA STEM

OIL	RISE IN CM. PER HOUR
Castor oil	0.05
Olive oil	0.50
Linseed oil	2.00
Oleic acid	0.75
Linseed fatty acids	1.00

It will be noticed that, contrary to SCHMIDT's findings, the fatty acid in the pure state did not rise as rapidly as did the neutral fats of these fatty acids. Also, the more viscous of the neutral fats did not rise as rapidly as did the less viscous. The fact that the free fatty acids did not rise as rapidly as the neutral fats might perhaps be due to their chemical properties in part, and to a different force of adhesion resulting from the chemical difference; but the reason for this difference in behavior is not clear. It may be mentioned here that these fatty acids, when applied to leaves of geranium, tomato, begonia, etc., kill the tissue with which they come in contact within twenty-four hours, while the neutral oils do not. Small amounts of neutral oil applied to one side of the leaf did not injure it, although large amounts naturally clogged all the openings of the leaf and injury resulted.

Most important, of course, was the intake of the oils by the cells. The fatty acids entered the cytoplasm from the intercellular spaces and appeared in the cytoplasm in the form of small droplets, which could be distinguished from those just outside of the cell by SCHMIDT's method of plasmolyzing the cell or by finding the oil in cells which happen to have rotating protoplasm. So far as intake of free fatty acids was concerned, therefore, SCHMIDT was evidently correct, but it was found that linseed oil with a free acid content of less than 2 per cent penetrated the cell as well as the free acid. It was evident, therefore, that these results again failed to check with those of SCHMIDT, who claimed that neutral oil did not penetrate

the cell. It seemed likely from this that the penetration was a physical matter, depending in part at least upon viscosity of the oil, and not a chemical process as he supposed.

The penetration of the cell wall by the oils being established, the next point to be investigated in the case for fat movement which SCHMIDT had made, was the soundness of the assumption that their passage from the intercellular spaces into the cell justified the conclusion that the fats could pass in a similar way from one cell to another. This point was tested on two bases: (1) Since the fat in the plant cells during translocation is in small droplets (emulsified), while the fats taken from the outside by the cells of the pea seedling are pure and homogeneous, the cases could not be considered at all comparable. Intake must be demonstrated from the emulsion state, with the continuous phase a water solution. (2) Since the seedlings used in SCHMIDT'S experiments were starved and etiolated, an abnormal condition, it should be ascertained whether any alteration in the condition of the walls bordering on the intercellular spaces might have been brought about, such as degree of saturation, that would be different from the conditions existing in the germinating fatty seed.

In order to test the first point in question, emulsions of linseed fatty acids were prepared with water, the fatty acids forming the dispersed phase. After three days' standing in this emulsion, the pea seedlings showed no intake whatever of the fat by the cells. Seedlings were incised in the usual manner, and the emulsion brought into contact by means of a jacket slipped over the pea stem. No rise of fat in the intercellular spaces or intake of fat by the cells was detected. It appeared, then, that when the plant conditions were approximated in this matter, the fats failed to penetrate to the interior of the living cell.

On the second point a suggestion was given by the results on the first, namely, that the presence of water might interfere with the intake of fat from the intercellular spaces; that is, that the penetration of the cell walls by the oils was due to a water deficit. It is well known that oil will not pass through cellulose membranes that are saturated with water, but will penetrate as the water dries out. SCHMIDT went into this phase very thoroughly, testing also some

plant membranes, with the same general results. A very definite suggestion that this might be the explanation of the intake of the oil lay in the fact that the cells which took up the fat were located in the periphery of the stem. The epidermal and subepidermal cells showed the most penetration. The intercellular spaces are large, and they connect with the outside through the stomata. This connection is easily demonstrated by placing a drop of colored oil on the pea stem and noting that it rises up through the internal spaces after half an hour. Consequently, we should expect that this etiolated, uncutinized, delicate seedling would lose water readily, especially from the cells nearest the epidermis. In fact, when the stored foods have been exhausted and the plants are exposed to a dry air in the laboratory, the epidermis of the stems soon wrinkles with water loss. Probably the starved condition interferes with its normal water holding powers.

With this situation in mind, the following test was made to discover whether water deficit played any part in the results obtained by SCHMIDT. Etiolated pea seedlings were placed under a bell jar and the soil was soaked with water; the peas and the air were soon approximately saturated. Another group of seedlings, in dry soil, was placed under the current from an electric fan. Then the seedlings were incised and the oiled strips of filter paper inserted. After two and four hours the amount of rise shown by the oil was noted in each case, and the cells were examined for penetration. In the seedlings saturated with water, the rise of the oils in most cases was zero, and of course then there was no penetration. In the few cases where some rise occurred, there was no detectable intake of oil by the cells. The increased drying effects of the air current increased the rate of ascent of the oil, however, and facilitated its penetration. Seedlings that were cut off, and, after injecting with oil, laid out to dry, showed a relatively rapid movement of the oil, while cut-off seedlings that were kept in water with a large test-tube inverted over the top permitted little or no rise. In this manner it was experimentally demonstrated that the intake of oil by the living plant cell, obtained by SCHMIDT, was due to an unnatural drying out of the cell walls, a condition not obtained in the tissues of the germinating fatty seed through which the fats have been supposed to pass as fats, if

indeed it obtains anywhere in the normal plant. The main point upon which the case for fat movement in the plant has rested, therefore, appears to have been reached through an abnormal condition of water deficit and through the use of oil in the pure instead of the emulsified state. Neither of these conditions exists in the plant in those tissues through which the oil has been thought to move.

Another point on which SCHMIDT had in a measure based his conclusion that the fats do move in the germinating fatty seed, was the fact that he found no sugar or starch in the endosperms of some of the fatty seeds during germination. When this point was investigated for the purpose of checking his results, it was found that there are large quantities of non-reducing sugar in the endosperm of germinating castor bean, as well as in hemp. A reducing sugar was found in flax endosperm during the germination phase. Others were not tested, but it was evident that SCHMIDT'S report was in error, possibly because he may not have tested for non-reducing sugars.

Finally, there remained the oldest and most frequently cited evidence for fat movement in plants, the fact that in germinating fatty seeds oil is found in the hypocotyl, and, as SCHMIDT demonstrated, this oil in the new tissue is similar in composition to that of the storage region. The fact that oil was stored in the seed, and later, during germination, appeared to be present in the new organs, was taken as evidence that it moved from the point of storage to the point of utilization in the fatty state. This is not a logical conclusion. No one believes that starch grains move from cell to cell as starch, and yet a perfect case for starch grain movement could be based on this principle. Various starchy seedlings show starch in the hypocotyls and other new tissue, and there are even cases, such as the storing of foods in ripening seeds, which furnish the appearance of a gradient of starch flowing toward the center of storage. Examination of any of the fatty seeds available showed considerable fat in the hypocotyls during germination, but this could not be taken as evidence of movement as fat. Similarity in the composition of the fat in the seed and the hypocotyl again has its parallel in the similarity in the composition of the starch in the seed and in the new organs; yet we do not conclude from such comparisons that starch moves as starch. The plant has its "patterns," and the like protoplasm of the different regions merely follows these.

SCHMIDT'S theory of "soaplike linkages" operating in the intake of fats by the cell should be mentioned, in view of the fact that it is believed by some today that fatty acids may be transported as soaps. For this to be possible the tissue must be alkaline, in order that the soap may not be decomposed. Seedlings of a number of the fatty seeds available were tested microchemically with the LaMotte series of indicators, and in all cases all the new tissues were found to be acid. The highest P_H found was 6.8, and in most of the tissues it was below 6.2. These determinations are accurate to within 0.2 in the P_H reading, which is sufficient for this problem. Furthermore, it seems to be the only method available by which local H^+ ion concentration may be determined in the tissues. Table III gives the ranges found in the hypocotyls of the seedlings tested. The root tips, both primary and secondary, were nearest the neutral point but were not above 6.6.

TABLE III

RANGE OF H+ CONCENTRATION IN HYPOCOTYLS

SEED	RANGE OF P_H
Peanut (fatty)	5.4–5.8
Sunflower (fatty)	5.2–5.6
Castor bean (fatty)	5.2–5.6
Hemp (fatty)	6.2–6.6
Pea (starchy)	5.6–5.8

The phloem of these tissues was examined with special care, in view of the fact that it has been said to be alkaline (4), but it was no exception to the general rule of acidity in these seedlings. Under these conditions, of course, it would be difficult to conceive of the existence of, and still more the movement of, soaps in the plant.

This concludes the report of the results obtained by attempting to repeat work previously done on the subject of fat movement. When the past evidence in favor of the transport of fats as such is repeated and reinterpreted, it is found that there is not even a legitimate suggestion that such movement occurs in the plant. In fact, there is no acceptable evidence as yet reported on either side of the question. For this reason further experimentation was carried out on the problem, with a view to finding some substantial and definite evidence leading to a solution. The results given here are not as extensive as might be desired, nor is the attack on the problem

as varied as might be, were the problem more easily approached. All evidence obtained, however, whether reported or not, pointed in the same direction, and only that is reported which is thought to be incontestable in its application.

The first attack on the question was made from the standpoint of the respiratory quotient, the object being to determine the type of food used by means of the type of respiration of the new tissue. It is well known that when sugars are being respired, a definitely higher CO_2/O_2 quotient is obtained than when fats are being respired. Theoretically the quotient ought to be about 1.0 for the carbohydrates in normal conditions, and about 0.67 for fats, but under the rapid growing conditions that prevail in germination the quotient is lower than normal, probably due to the formation of organic acids. If any considerable portion of the fat of the seed were to be moving into the hypocotyl during germination, then the tissues of the hypocotyl would be digesting and respiring fat, in part at least, and the respiratory quotient would accordingly be lowered. Whereas, if the hypocotyl received only sugars, that is, if the fat of the seed moved into the hypocotyl as sugar, we should expect to find a respiratory quotient of the same order as in the hypocotyls of starchy seeds. Even though the sugar was only the transit form, and was converted back to fat immediately upon reaching a certain point in the new tissue, the quotient would be a carbohydrate quotient, since the temporary storage of the sugar in the form of fat would be cancelled out or balanced off by the corresponding transformation of this temporary fat back to sugar for utilization. In short, if the carbon stored as fat in the seed should move as sugar into the hypocotyl, it does not matter what changes back and forth it may go through before utilization, so far as the effect on the respiratory quotient is concerned; a quotient should be given of the same order as that of the starch-storing seed.

The method and apparatus used in determining the respiratory quotient of this material are modifications of those used by RHINE (3). The bottoms of three wide bottomed pint fruit jars were covered with wet cotton, and fitted with rubber stoppers, in which were set a mercury manometer and an outlet tube covered with heavy rubber tubing and closed with a screw clamp. The first jar was left with

only the wet cotton, as a check on temperature and barometric changes. The second was fitted with a small flat vat holding 10 ml. of 20 per cent NaOH, and around this vat the seedling material was laid. In the third was placed a like amount of plant material. In the second and third, the seedlings were covered with moist cotton, and all the jars were effectively sealed by a heavy application of sealing wax, completely covering the stopper and the connections with the tubing. The chambers were then placed in a water bath and allowed to come to the temperature of the bath before closing the outlet tubes. A water bath is not necessary with this method, however; it is merely convenient.

In some cases the hypocotyls were cut off, and in others the attempt was made to inclose the storage region or seed proper in a thick coating of beeswax or paraffin, and thus smother its respiration so that it would not affect the air of the chamber. Other substances were used also, but in general the results were best when the hypocotyls were simply cut off, paired carefully, and one lot of 50 or 100 put in each of nos. 2 and 3. When the seeds were coated there was danger of the coating being imperfect, and allowing the respiration of the storage region to affect the reading; but it must be remembered that any such error would throw the readings in the direction that would have opposed rather than favored the conclusion drawn from them. The results of these determinations are stated as respiratory quotients, and, since jars of equal volume were used, could be computed from the manometers. The data are given in table IV.

The very considerable variation in the results is due to variety in the tissues used; it is not possible to get exactly similar conditions in the samples used in different tests, and the value of the data will necessarily depend upon the number of determinations made. The average is the significant figure; the range of experimental error lies within $\pm .025$, although the variation of the tissue is greater than this figure. There are two sets of readings from fatty hypocotyls giving a quotient of 1.0 which are too high to be typical. While they would be favorable to the conclusion that is drawn from these data, they should not be included in the averaging of the results. Also, quotients as low as 0.66 have been obtained on pea (starchy) hypo-

cotyls, but these are unusual, and, though they would favor the conclusion based on the general data, have been eliminated. Other-

TABLE IV

RESPIRATORY QUOTIENTS ON HYPOCOTYLS OF FATTY AND STARCHY SEEDS

NAME	AVERAGE LENGTH (CM.)	TREATMENT	NUMBER OF READINGS	RESPIRATORY QUOTIENT
Fatty seeds				
Cotton 1	4	Cut off	2	0.80
2	4	Coated beeswax	4	0.73
3	3	Coated paraffin	2	1.00 (disregarded)
4	3	Coated paraffin	1	1.00 (disregarded)
Sunflower 5	3	Coated beeswax	3	0.90
6	3	Coated beeswax	3	0.75
7	4	Coated beeswax	2	0.74
8	4	Cut off	2	0.75
9	4	Cut off	2	0.70
10	5	Cut off	1	0.77
11	4	Cut off	1	0.82
12	5	Cut off	3	0.75
Average of total readings (except 3 and 4)				0.770
Starchy seeds				
Pea 1	4	Cut off	2	0.70
2	3	Cut off	2	0.66 (disregarded)
3	6	Cut off	2	0.85
Wheat embryos 4	1.5	Cut off	2	0.77
5	2	Cut off	2	0.78
6	2	Cut off	2	0.75
Barley embryos 7	4	Cut off	3	0.74
Buckwheat 8	3	Cut off	4	0.80
Average of total readings (except 2)				0.775
Respiratory quotients on cotyledons alone				
Sunflower 1	3 days old	Cut off	1	0.33
2	4 days old	Cut off	1	0.58

wise all the results that are free from errors in manipulation have been averaged.

It is apparent from the evidence of respiration that the food supplied to the hypocotyls by the storage region of fatty seeds reaches them in a form yielding the same order of respiratory quotient as

does the food supplied by the typically carbohydrate storage seeds. This is commonly understood to be sugar, and the great amount of sugar present in seedlings of the starchy type is considered as indication that this is the transportation form. It would seem, therefore, that fat could not be moving into the hypocotyl as fat, at least in such quantity as to affect the respiratory quotient.

The problem was then attacked from another angle, that of gradients. If it were possible for fats to move in the insoluble state (as droplets, fine emulsions) from the storage to the growing point of the hypocotyl or root tip, we might expect to find them in a "diffusion" gradient of concentration from the source out to the point of utilization. That is, the movement of these droplets could hardly be rapid in any case, and in view of the rapid rate of growth it seems reasonable that the movement of fats as such would provide a gradient of decreasing concentration going toward the region of active growth. To secure evidence on this point, both microchemical observations and quantitative determinations were made on hypocotyls of fatty seedlings. Using the microscopic methods with Sudan III, the hypocotyls of cotton, sunflower, squash, cocklebur, castor bean, peanut, and flax, taken when 1–6 cm. in length, were studied. In no case was there a gradient from the storage region to the growing tip. Strikingly to the contrary was the fact that in every seedling examined there was a distinct gradient in the other direction; that is, the amount of fat increased with approach toward the tip of the hypocotyl. In fact, the tips were heavily laden with fat, although they were undergoing rapid elongation.

The results of the quantitative determination of the fats are given in table V. They represent the ether extract, and are stated as percentage of wet weight, since this is the most accurate basis for such a comparison, approaching most closely to a volume basis. The hypocotyls were severed from the seed just outside the seed coat, and were divided into three parts, tips, middles, and bases. Care was taken to get rid of all adhering moisture without losing water by evaporation. The cut parts were kept in closed weighing bottles in order to prevent loss of water.

It might be thought that there could be a local channel through the hypocotyl in which the fats might move, and yet not be present

in such quantity as to register a gradient. The solution of this question lay in the microscopical technique. No such channel was detectable, although fine droplets of fat were clearly evident in the cortical parenchyma. These were fewer in the middles and bases than in the tips, and as the quantitative figures on squash show, there can be as much as twelve times as much fat in the tip as in the base, although the base is close to the fat storage center.

A third approach to a solution of the question of fat movement was made through a thorough study of the new tissues in which oil droplets were found, using the common fat stains, Sudan III, Scarlet Red, etc., with a view to seeing whether any test for oil could be obtained in the walls of the cells through which the droplets would have to pass if such movement occurred. It was thought prob-

TABLE V

PERCENTAGE OF ETHER EXTRACT IN PARTS OF FATTY HYPOCOTYLS

SEEDLING	PERCENTAGE ETHER EXTRACT ON WET WEIGHT BASIS		
	Tips	Middles	Bases
Cotton	5.75	2.24	1.33
Sunflower	5.31	1.34	0.66
Squash	2.59	0.60	0.22

able that if fat were found in quantity in two cells that are adjacent, and if this fat were passing from one cell to another, there would be some fat necessarily in the walls "en route," in some instances at least. The tissues through which the carbon stored as fat in the seed have to pass in the seedling were carefully examined, both in cross and in longitudinal section, but no evidence of fat passing through the walls was seen, and no staining of the wall with fat stains was obtained. This method of attack cannot be stated very effectively, and the results cannot be put very convincingly, but it should be considered as more evidential than any point yet made, following in importance only the statement of results under the next method of approach. It should be mentioned here that the fatty compound obtained from cell walls by HANSTEEN-CRANNER (1) was obtained only after considerable hydrolysis, and its fatty character could hardly have been present before hydrolysis; in fact, the walls did not stain with fat stains.

Finally, the question was considered from a physical standpoint, the mechanics involved in fat movement. Since the hypocotyls grow downward the fat would have to move downward to reach the tip, where most of it is found. In view of the relatively high viscosity of the cytoplasm, especially in the first centimeter of the tips, this becomes a questionable proceeding, no matter how small the droplets, so long as they are not in true solution. The most important point in this connection, however, should be the relative densities of the fat and the cytoplasm down through which it would have to pass. The cytoplasm, being a hydrosol, can safely be assumed to have a specific gravity greater than 1.00; probably it is much greater, as solutions go. Extracts of the seedlings were made from different parts of the hypocotyl, and while there was not enough obtained to determine accurately the specific gravities of the various extracts, in every case they were lighter than water. They were less dense than egg white, and various concentrations of gelatin. There can be no doubt that they were lighter than the cytoplasm. It is true, as has been found in the course of some unpublished work on the oxidation products of fatty acids, that the highly unsaturated fatty acids readily become heavier than water through oxidation. The extract from the tips seemed most to resemble the oxidized oil, but even this did not become as heavy as water. Fat movement, then, would have to take place against the pressure of a denser medium, if it occurs. It will be remembered, of course, that SCHMIDT'S movement of oils up and down the etiolated pea seedling was a movement of homogeneous oil, not an emulsion of oil in water, and that the movement was in the intercellular spaces, not through the living cells in any case.

Summary

1. It was found, as SCHMIDT had reported, that liquid fats would rise in the intercellular spaces of etiolated pea seedlings and would enter the cell. Contrary to SCHMIDT, however, it was found that neutral fats rose more rapidly than free acids in certain oils, and furthermore that neutral fats entered the cells from the intercellular spaces. SCHMIDT'S theory of fat intake was thus shown to be incorrect.

2. It was shown that while the liquid fats pass into the cell from the intercellular spaces of the pea, this cannot be taken as analogous

to the situation existing in plants in which fats have been thought to move from cell to cell. It was shown that the intake of the fat was due to a water deficit in the walls of the starved etiolated pea seedlings, when they were exposed to unsaturated air, and that when well supplied with moisture in air and soil they would not allow either the rise or intake of oils.

3. SCHMIDT'S report that the endosperms of fatty seeds did not have sugar during germination was found to be incorrect, and, therefore, this evidence is not admissible in support of fat movement in plants.

4. The presence of oils of similar composition in the new tissue and the old was shown to be of no significance in this connection, since the same condition prevails with respect to starch in starchy seedlings.

5. SCHMIDT'S theory of "soaplike" linkages forming and aiding in fat movement is shown to be untenable, in view of the P_H values found in the seedlings investigated. This point also covers the question of movement of fatty acids as soaps, making the existence of soaps in the cell seem impossible.

6. By determining the respiratory quotients on hypocotyls of fatty and of starchy seeds during germination, evidence was obtained that the fatty seed hypocotyls were being furnished carbon in the same state of reduction as were those of the starchy seeds. The logical inference is that that form could not be fat.

7. If fats were to move as such a gradient of decreasing concentration might be found in the region of most active growth. The contrary is the case, both microscopical and quantitative data showing that a steep gradient exists in the opposite direction, increasing with approach toward the tip of the hypocotyl.

8. Cell walls in tissues through which the fats have been thought by some to pass, and in which fatty droplets may be found, have been examined for fats "en route." No evidence of such movement was found.

9. Since the oils would have to pass downward through several centimeters of denser viscous medium, to say nothing of the admitted impenetrability to oil of the water saturated cellulose membrane, the fat movement theory, from the standpoint of the physics in-

volved, would have to be given up, at least until some good positive evidence that such movement can and does occur is presented.

10. All evidence in favor of fat movement in plants that has been considered and reinvestigated has been found incorrect in fact or interpretation. The further evidence bearing on the problem, presented in this paper, favors the view that all the fat stored in the fatty seed is, as we have known most of it to be, first converted to sugars before being transported.

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PLANT SUCCESSIONS ABOUT DOUGLAS LAKE, CHEBOYGAN COUNTY, MICHIGAN

FRANK C. GATES[1]

(WITH THREE FIGURES)

Introduction

During the period between 1911 and 1925, the writer has been making a detailed study of the region about Douglas Lake, the location of the Biological Station of the University of Michigan. During this study special attention has been paid to the successions between the various plant associations. The present paper is the result of that study.

Douglas Lake is located in the west-central part of Cheboygan County, at the northern end of the lower peninsula of Michigan, midway between Lakes Huron and Michigan, at an elevation of about 712 feet above sea level. The region which has been more thoroughly studied includes the area from Brutus east to Topinabee, north around Mud Lake and west to Levering, then south, with an extension west of Pellston, to Brutus (fig. 1). In addition, the region along Lake Michigan from Cecil west to Temperance Point has received special study. The surface soil is entirely of glacial origin.

According to the scheme of life zones drawn up by MERRIAM,[2] the Douglas Lake region would be considered as belonging to the Canadian part of the boreal zone, although it actually partakes of transition zone characteristics as well. According to the moisture-temperature index of LIVINGSTON,[3] the 3300 calculated for Alpena, a short distance south, indicates an area of relatively low plant development. Agriculturally, therefore, the region would be considered

[1] Paper no. 205 from the Botanical Department of Kansas State Agricultural College. For the higher plants of the region see GATES, F. C., and EHLERS, J. H., An annotated list of the higher plants of the region of Douglas Lake, Michigan. Michigan Acad. Sci., Arts and Letters 4:83–284. 1924.

[2] MERRIAM, C. HART, Life and crop zones of the United States. Bull. 10. Div. Biol. Survey. 1898.

[3] LIVINGSTON, B. E., A single index to represent both moisture and temperature conditions as related to plants. Physiol. Res. 1:421–440. 1916.

as specially favorable to wild berries, currants, blueberries, blackberries, and cranberries; and in more favorable places suitable for Irish potatoes, turnips, beets, the more hardy apples, and cereals such as wheat, oats, barley, rye, buckwheat, and also timothy.

From the phytogeographic standpoint, the Douglas Lake region is located in the transition zone between the northeastern coniferous forest province and the central or deciduous forest province. While the region is near the northern part of the transition belt, conditions are becoming more favorable to the deciduous forest province.

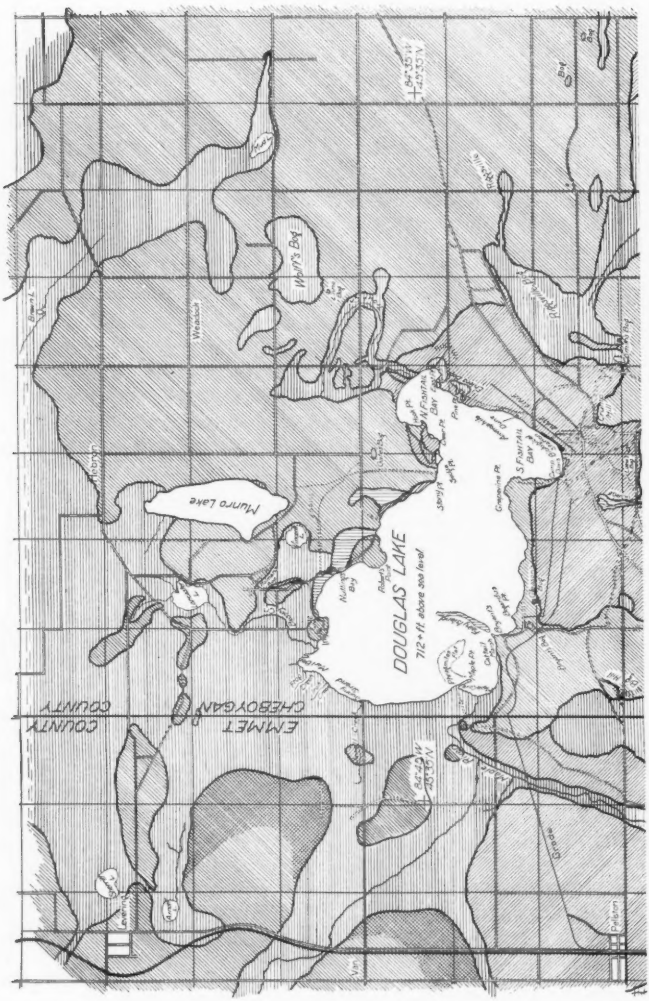
Factors

The Douglas Lake region is an area in which the climatic factors have been and are now favorable for the development of trees as a ground cover for the whole region. The temperature is moderate, the rainfall likewise is moderate, but amply distributed throughout the year. Snow is usually abundant, and remains on the ground for a long time.[4] The soil is prevailingly of a sandy type. This may be mixed with gravelly or clayey material in the glacial moraines, but is nowhere a heavy loamy soil. In the lower parts of the region where bogs have developed the soil is largely of organic nature. Fire is probably the most important single factor in the region at the present time. It has operated extensively and repeatedly in the region since lumbering.

History of vegetation

Following the withdrawal of the ice, the different parts of the area became vegetated until, at the time of lumbering in the 1870's, three conspicuous types of vegetation were present. The poorer or sandy uplands were covered with pine forest (*Pinus Strobus* and *P. resinosa*), grading rather sharply into beech-maple forest on the better soils of the uplands. The lowlands were cedar bogs (*Thuja occidentalis*). Depressions which filled with water became lakes, and aquatic vegetation was developed in and bordering them. The region was predominantly one of extensive forest.

[4] For the detailed figures of meteorology of the Biological Station see GATES, FRANK C., Meteorological data, Douglas Lake, Michigan. Papers of the Mich. Acad. of Science, Arts and Letters, 4:475–489. 1924, and other papers referred to there.



With the lumbermen came the removal of the forest, first from the pineland, and later, even up to the present time, the beech-maple forest (*Fagus grandifolia-Acer saccharum*). Areas that were

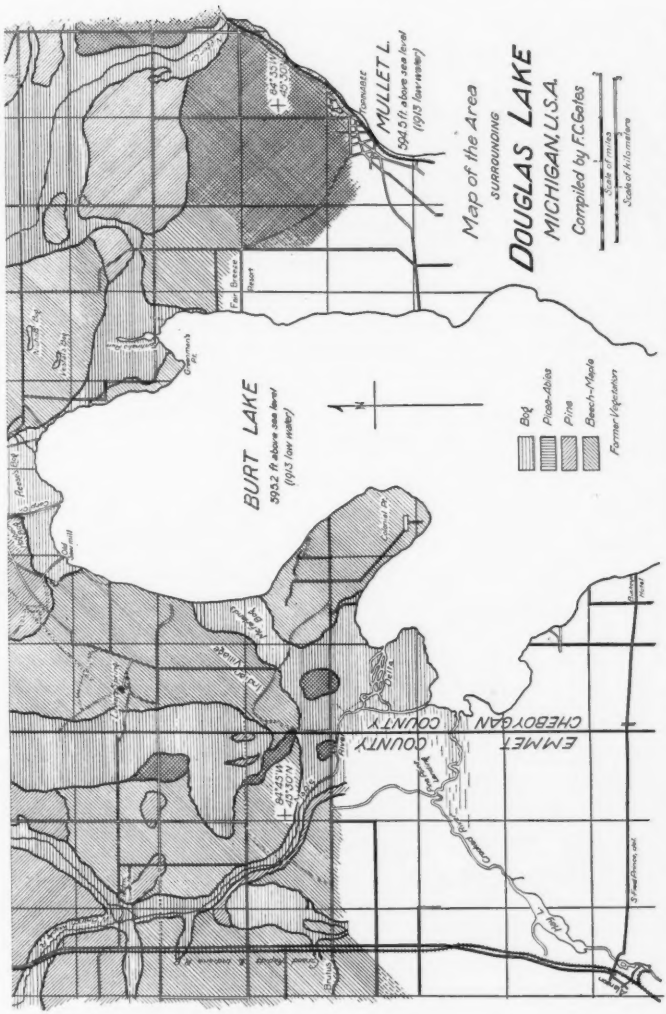


FIG. 1.—Map of area surrounding Douglas Lake, Michigan, showing approximate distribution of principal types of vegetation previous to era of lumbering.

lumbered were nearly always burned and usually burned repeatedly. The cedar bogs were largely cut and more or less burned, but, as bog conditions are much less favorable for extensive fires, such areas

are not so badly damaged. The immediate result of the clearing and burning was the installation of a new vegetation cycle over widespread areas. Fireweeds (especially *Epilobium angustifolium*) came first, and later shrubs and trees. Areas that have been repeatedly burned are now largely covered with aspens (*Populus* spp.). This type of vegetation is favored by the occasional fires, at the expense of the pines, beech, or maples.

Description of region

At the present time the region includes lakes and ponds in the depressions in which water accumulates, with rivers or smaller streams connecting them. In each there are appropriate types of aquatic vegetation, showing various stages of development from the deep water to the shore. The shore conditions around the larger lakes are those of the strand, and are subject to the action of storm waves and ice. Appropriate vegetation is very scanty because of the thoroughness of the ice work, and the fact that replenishment of suitable plants is not possible from the immediately surrounding vegetation, which is present under sharply different conditions. On a few of the most exposed shores, where opportunity is afforded for sufficient wind action in the right direction, small dunes are present behind the strand. Where shores are relatively protected, as in the smaller lakes or ponds, or on the lee sides or in coves in the case of the larger lakes, marsh development usually replaces that of strand, and leads rather abruptly into the distinctly land type of vegetation. Occasional seasons of high water are accompanied by such severe ice work that the region is not favorable for the development of lowland woods or bogs down into the water. River banks vary in vegetation, depending upon whether the bank is steep or low, and upon the character of the stream, whether swift or slow and whether subject to much ice work or not, and whether subject to human interference. Steep banks are likely to have a characteristic vegetation and different from the immediately adjacent area. Low banks where the streams are slow may not differ much from the adjacent area, except when there is a very radical change of soil type or some other local condition.

Land subject to overflow or spring flooding is vegetated with

lowland forest or in stages leading up to that stage, as willows, dogwoods, and similar thicket plants.

Rock shores and rock ledges are not present in the region close to Douglas Lake. Those washed over by Carp Creek in its descent into Lake Michigan at Cecil have a vegetation but little different from stream bottoms of other types.

On the lowland surrounding or adjacent to several of the lakes we find the cedar bog forest in considerable quantity, or stages of development which under normal conditions will sooner or later lead up to it, as for example, *Chamaedaphne*, *Carex lasiocarpa*, and *Larix laricina* associations. On the better land, in which there is more or less clay mixed with the sand which prevails in the region, we find a few virgin beech-maple forests, also several other forests in which selective cutting has taken place, leaving the poorer and smaller trees and modifying the ground conditions materially with the admittance of a great deal of light to the soil. There are many similar areas in which more or less burning has taken place and the vegetation in consequence represents various stages and mixtures of aspens and hardwoods, tending toward replacement of the beech-maple forest; also there are many areas in which the burning has been so severe that aspen dominance is complete and it is rather problematical whether the beech-maple forest will come back; and finally there are many areas in which the forest has been completely removed and the land used for agricultural or grazing purposes, in which case there is no chance of the return to the beech-maple forest, so long as such conditions are maintained. On the poorer soils of the uplands, which are only too evidently little but sand, one no longer finds any original pine forest, but finds many and extensive areas entirely dominated by aspens in various stages of development, depending upon the time of the last fire in that particular area or part of an area; also there is a number of areas in which seeding of pines is taking place relatively rapidly and several to many young pine trees are to be found among the aspens, with every indication of sooner or later replacing them entirely in the vegetation. There are a few areas in which the replacement has taken place and where we now have a young pine forest with trees 6–9 m. high and 20–30 years of age in areas somewhat protected from burning by their loca-

tion with respect to ridges or roads; and there are many areas in which aspen dies only to be replaced by aspen, as previous fires have completely destroyed any seeding pine trees and the nearest such are somewhat too far away even for stray seeding.

Cultivation

There is no longer any land used for agricultural purposes in the close proximity of the Biological Station, although one can find such at the edge of the region. Areas grazed by domestic animals are not sufficiently numerous to be studied satisfactorily. Actual farming is conducted on a small scale in a few places in the region. It consists largely of truck gardening for the few families and for the resorts of the region, together with some dairy farming. The horticultural possibilities of the area are slight, and are most likely to consist of apples and cherries. Small berries are quite abundant, but usually the supply is obtained from natural growth and not by cultivation. Such include particularly blueberries, strawberries (also the cultivated kinds), red raspberries, blackberries, dewberries, and cranberries. Good pasture land close to the Station is rather rare, but adjacent pasture land in very good years is excellent.

Associations

Forty-three associations may be recognized in the area. In their delimitation special attention has been given to the floristic composition, but in some cases, notably on the dunes, the physical factors of the habitat are so important that they need to be taken into active consideration. In other cases certain plant groups act apparently independently of the habitat and are found in places where they would not normally be expected, indicating that one cannot lose sight of the plants themselves, even though a classification based on habitat alone is much easier to construct.

In the beginning there was no such thing as an association. As disseminules get into a new area and develop, plants appear here and there, depending absolutely upon the growth of these disseminules, although the exact location may be a matter of the operation of one or more physical or chemical factors. As such plants usually grow separately and without keen competition, the result is a very open

assemblage. As these grow more closely together by becoming larger, by vegetative propagation, or by additional individuals coming into the interstices, the conditions become different. In the first place the crowding leads to the elimination of plants that cannot withstand such conditions, whether from lack of food or light, or because of the soil or other conditions. This results in the elimination of the plants that are unsuited and the spread of the plants that are there and are suited. The whole result is the condition which we call an association; in other words, a group of plants that live together under the conditions present.

In most cases the life of an association is interfered with by the invasion of disseminules from another association. If these do not find conditions suitable for growth, there is no possibility of succession. On the other hand, if conditions are favorable for the development of these invading disseminules but the plants do not crowd out the plants already there, there is merely an addition to the association. Such additions may merely add complexity to the vegetation, or they may serve as nuclei around which successions may take place under more favorable conditions. When the disseminules find suitable places for growth and development, however, and become plants which in one way or another dominate over the plants that were there, succession has taken place.

Succession

The process of succession is a natural one in which there are many contributing factors. The actual cause is and must of necessity be invasion and growth of a new order of plants in an area previously occupied by a group of plants. There may be a number of contributing or limiting circumstances, however, which many authors refer to as actual causes. The contributing circumstances which favor succession that are particularly active in this region are changes of water level, both permanent and fluctuating, formation of beach pools, flooding, ditching and cultivation, lumbering, changes in the reaction of the water, changes in the evaporating power of the air, running water, erosion, humification of the soil, and the work of certain insects and changes of light. Most prominent among the factors tending to prevent succession, or to check it

after it is started, are fire and the work of water in the form of streams, waves of lakes, and when frozen as ice.

Genetic series

By genetic series is meant a series of associations related in a continuous succession. Under normal circumstances a genetic series should begin with its lowest, and consequently ecologically simplest associations, and proceed through ecologically more complex associations until the climax for the given conditions is reached.

The duration of any genetic series depends entirely upon the duration of suitable conditions. In extreme cases a series may be completed within a year or two. Other series have been running for ages. It is possible for one series to change to another if conditions favor such a change and the necessary plants occur. Physiographic changes in the land frequently favor changes from one genetic series to another, as may easily be shown in the change of stream courses. Each series is usually a physiographic or biotic unit in the whole series of successions. It is separated from the whole series because of some striking peculiarities which make it distinct and consequently convenient to handle. A group of series fits into a complete discussion of successional relationships of the associations of any region.

In taking up genetic series three main groups can be recognized, two of which, designated as xerarch and hydrarch, are natural series depending upon the water content of the soil, and might together be designed as feralarch[5] series. The third group (hemerarch) contrasts in being brought about by cultivation. Taking up first the hydrarch series, fig. 2*C* shows the associations that are normally found in the change from lake to dry land. The line running from lake to dry land is the condition of open lake with waves where plants are not present. The upper part of the figure shows the condition of the lake in relatively waveless parts, free from the severest ice work except at the very shore itself. The lower part of the figure brings out the condition in waveless parts of the lake, where there is continuous vegetation from the lake into the dry land associations. The situation in ponds and pools is similar to this last case.

[5] GATES, F. C., Hemerarch and feralarch, two additional terms in ecology. Science 61:260. 1925.

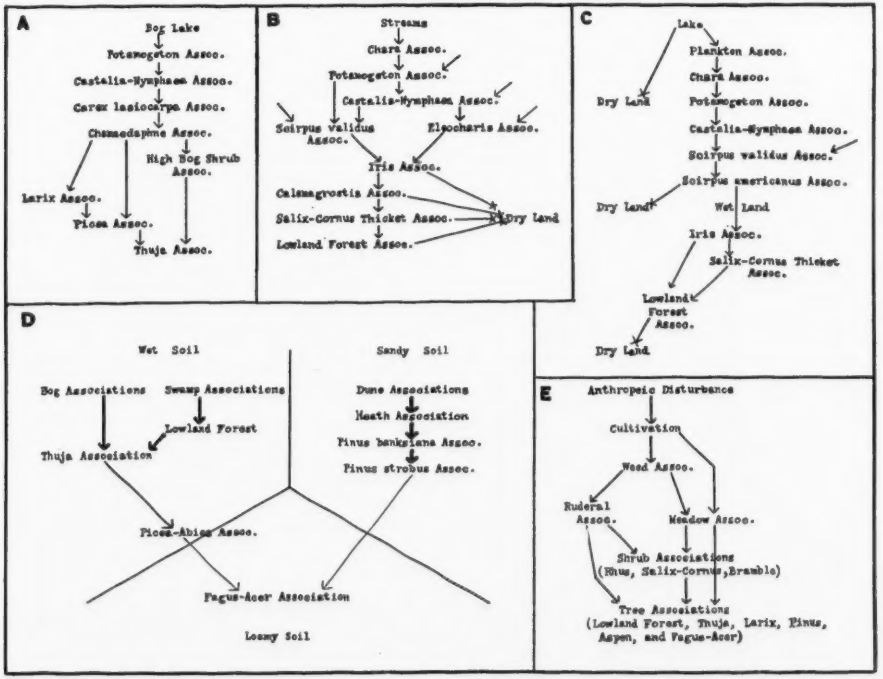


FIG. 2.—Diagrams showing usual successional relationships between associations of most important genetic series in region of Douglas Lake, Michigan: *A*, hydrarch series in bog lakes; *B*, hydrarch series in streams (*X* in place of arrowhead indicates juxtaposition); *C*, hydrarch series in the lakes; *D*, land series (successions indicated by light arrows are rare and involve profound changes of environment); *E*, relationships involved in return from hemerarch to feralarch series.

Lakes which form in relatively undrained areas show a different type of succession, which is exhibited in fig. 2 *A*. The succession proceeds from open water associations, through a mat developing over the water, and then into ericaceous shrubs and finally coniferous trees. The conditions in eroding streams are shown in fig. 2 *B*. The inception arrows here indicate that the series may begin in several places, and the crosses at the end of lines in place of arrows indicate that there may be an abrupt junction of the aquatic vegetation with that of the dry land, as for instance in the case of a bluff.

Land groups

Among the land groups there are three distinct conditions, which are collectively shown in fig. 2 *D*. The starting point from sandy soil is indicated in the upper right. In the upper left is shown the transition from lowland to upland. In this part of the figure the lowland forest and the *Thuja* association are given as the end members of wet ground genetic series. These associations may persist through the change from wet ground to conditions which partake very largely of upland, but whose vegetation has not yet had time to change to a true upland type; or they may persist in places where succession is impossible due to occasional flooding which continues to kill out such members of the upland association as have started during dry periods. In the lower part of the figure is shown the loamy soil on which the beech-maple association, which is the regional climax, represents the intrusion of the central deciduous forest province into the region. At present this is the one association of that province well represented in the region. The lines separating these groups indicate that before a change is made from one genetic series to another, a conspicuous change must be made in the habitat. For instance, the progressive drying, due to lower water tables or to the blowing in or washing in of material into low wet ground, builds it up sufficiently for the beech-maple forest to enter. Likewise the humification of sandy soil gradually permits the invasion of the beech-maple forest. The upland has been disturbed by lumbering, slashing, fire, and cultivation.

Summing up these types of disturbances in fig. 2 *E*, the general relationships are indicated by the interrelation of the types of associations that may develop there.

Having taken up the usual series from the conspicuous standpoints that are present in the region, for those who are particularly interested in the summation of the matter in a single diagram, fig. 3 is

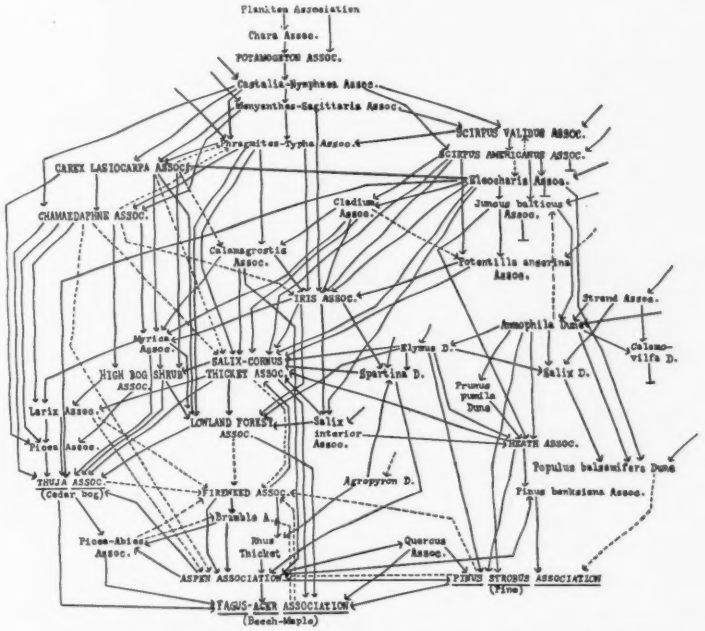


FIG. 3.—Diagram showing successional relationships exhibited between plant associations in region of Douglas Lake, Michigan: broken lines indicate secondary successions; solid lines, primary successions; an arrowhead, direction of succession, a cross line at end, that succession usually terminates here; an arrow from the open means that the association may start *de novo;* capital letters denote more important associations, while those underlined dominate the three principal soil types of region.

given. All facts with regard to succession between associations that have been observed in the region are here indicated. As in the other figures, the arrows point to the direction of succession; arrows coming from no association indicate the possibility of inception of a series; dotted lines represent the secondary associations following various disturbances. The three conspicuous soil types are indicated by underlining their climax associations. In interpreting a diagram of this sort, of course one must be careful to avoid the assumption

that every bit of ground in the region will go or has gone through all steps indicated here, as there are many cases where in given spots a complete series is never present, but steps may be jumped. For instance, the *Chamaedaphne* association may go to any one of eight different associations, although the usual case is indicated in the earlier diagram (fig. 2 *A*). As is readily seen in this figure, the vegetation of the region is very intricate, due to the great disturbances that have gone on in the past and which are far from being stabilized at the present time.

Summary

1. The Douglas Lake region is located in the northern part of the overlapping zone between the Northeastern Coniferous Forest Province and the Central Deciduous Forest Province in the extreme northern part of the Lower Peninsula of Michigan.

2. The former distribution of the most widely distributed plant associations is presented in a map of the region.

3. The intricate successional relationships between the plant associations which exist today are shown in six diagrams.

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TEMPERATURE AND RESPIRATORY ENZYMES OF APPLES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 352

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(WITH PLATE XI)

The recent interest in the marketing of food products has called attention to the lack of understanding of life processes that go on in these products. The early physiological reports on this group of plant structures are fragmentary and need revising, using modern methods. This is especially true in enzyme activity and the localization of elements, products, or processes.

THATCHER (15) carried out tests on diastase, invertase, tannase, esterase, protease, oxidase, and emulsin type enzymes in apples. He concluded that ripening changes were chiefly due to oxidases. OVERHOLSER and CRUESS (13) studied the oxidation system in Yellow Newtown apples in relation to the browning of apple tissue. They were able to separate an enzyme (peroxidase), an organic peroxide, and a tannin, using qualitative methods. Qualitative tests (using benzidine and peroxide) indicate that peroxidase is a prominent enzyme in apple tissue.

Respiration is one of the essential processes of living organisms. Early studies of this process, in the case of apples, were concerned chiefly with the respiration quotient. GERBER (5) made such a study, sealing the fruit in chambers. GORE (6) and MORSE (11) reported that respiration in apples followed Van't Hoff's rule, that is, it increased 2.9 and 2.38 times in these tests, with an increase in temperature of 10° C. Their tests were for short intervals, and include comparatively few measurements of respiration rates. KIDD and WEST (9) found that high carbon dioxide concentrations in the storage air injured the flavor of apples. They concluded that the gas supply surrounding the cells in apple tissue affects respiration products and the development of flavor. BURROUGHS (3) made very complete determinations of respiration rates in apples for a short

period following picking. He worked with a number of the winter varieties, Ben Davis, Baldwin, Wagener, Spy, and Wealthy. His records show an increase in respiration following picking with fruit held at 68.5° F. Early picked fruit had a smaller initial respiration rate than those picked later.

Methods

The work cited suggested a study of respiration rate from the time the fruit was picked until decay set in, with the hope of obtaining a general idea of the rate of life processes in ripening apples. The investigation was conducted during the summers of 1921 and 1922. A series of determinations was made on the summer varieties, Maiden Blush and Oldenburg, using two temperatures, 25° and about 0° C. A constant temperature was secured at 25° by using a Freas water thermostat, and at 0° by the use of a temperature box cooled by brine and electrically controlled. Both fruit and apparatus were inclosed in the cold box to reduce variations, but the temperature varied about 2°. The water bath did not vary more than 0.25°.

The fruit was secured from orchards in Michigan and northern Illinois. It was picked from a single tree in each case, carried to the laboratory, and placed under experimental conditions as soon as possible. The first determinations were made within twelve hours after picking for Maiden Blush samples, and within forty-two hours for the Oldenburg. All samples were run in duplicate. Each sample contained 1–3 kg. of fruit, and was kept constantly ventilated throughout the experiment.

Respiration rate was determined by measuring carbon dioxide produced, using the method described by GORE (6) as modified by KÜSTER (10). A standard solution of barium hydrate in two Reizet absorption towers was used to take up the carbon dioxide. Then the excess alkali was titrated against a standard solution of oxalic acid, using phenolphthalein as indicator. At the higher temperature, readings of carbon dioxide production were made daily, and at the lower temperature every day or two. Tables I and III show the amount of respiration for Maiden Blush and Oldenburg apples held at 25°, and tables II and IV present data on respiration of the same varieties at 0° C.

In every case the fruit was placed under experimental conditions as soon as possible after picking. A similar series of determinations was made for Winesap apples purchased on the market. This fruit

TABLE I

MILLIGRAMS OF CO_2 PER KILOGRAM HOUR AT 25° C.
MAIDEN BLUSH APPLE

TIME AFTER PICKING IN DAYS	SAMPLE *a*	SAMPLE *b*	AVERAGE
1	78.76	85.80	82.28
2	195.80	175.56	185.68
3	193.16	169.62	181.39
4	257.62	208.56	233.09
5	343.86	233.20	288.53
6	310.64	249.48	280.06
7	292.60	269.50	281.05
8	297.44	355.74	326.59
9	448.80	338.80	393.80
10	297.66	229.90	263.78
11	291.06	Decay started	
12	224.18		
13	221.54		
14	193.16		
15	191.62		
16	171.60		
	Decay started		

TABLE II

MILLIGRAMS OF CO_2 PER KILOGRAM HOUR AT 0° C.
MAIDEN BLUSH APPLE

TIME AFTER PICKING IN DAYS	SAMPLE *c*	SAMPLE *d*	AVERAGE
2	24.20	31.24	27.72
4	21.34	46.20	67.54
6	42.90	50.82	46.86
8	40.48	Discontinued	
10	7.48		
12	14.52		
14	20.90		
16	46.64		

had been in cold storage and was on display at the time of purchase (July 3). Samples were taken and handled as described for the Maiden Blush and Oldenburg apples. The data in tables V and VI indicate the rate of respiration in this fruit at 25° and 0° C. respectively.

Most apples are more or less bruised in harvesting and handling. Some determinations were made to discover whether bruising modified the carbon dioxide output. Specimens of Oldenburg apples were

TABLE III

MILLIGRAMS OF CO_2 PER KILOGRAM HOUR AT 25° C.
OLDENBURG APPLE

TIME AFTER PICKING IN DAYS	SAMPLE *a*	SAMPLE *b*	AVERAGE
2	55.88	47.52	51.70
3	75.02	75.46	75.24
4	59.84	68.20	64.02
5	60.94	68.86	64.90
6	62.26	63.14	62.70
7	54.56	57.20	55.88
8	48.62	58.96	53.68
9	53.90	53.90	53.90
10	52.14	50.38	51.26
11	53.02	52.80	52.80
12	.....	37.62	.....
13	46.86	45.76	46.20
14	37.18	35.42	36.30
15	40.48	43.34	41.14
16	41.36	38.72	40.04
17	22.66	41.58	32.12

TABLE IV

MILLIGRAMS OF CO_2 PER KILOGRAM HOUR AT 0° C.
OLDENBURG APPLE

TIME AFTER PICKING IN DAYS	SAMPLE *c*	SAMPLE *d*	AVERAGE
3	151.58	210.98	181.28
5	29.26	39.38	34.32
7	33.22	45.32	39.27
9	39.82	47.30	43.51
11	66.88	70.18	68.53
13	89.98	29.04	59.51
15	29.26	33.00	31.13
17	36.74	25.96	31.35

bruised about half way through the apple, but without visibly breaking the skin. Such severe bruising should give a maximum response, but without complications arising from increased accessibility of oxygen. They were tested only at 25° C. The results are shown in table VII.

TABLE V

MILLIGRAMS OF CO_2 PER KILOGRAM HOUR AT 25° C.
WINESAP APPLE

TIME AFTER PURCHASE IN DAYS	SAMPLE *a*	SAMPLE *b*	AVERAGE
1	35.42	38.72	37.07
2	29.92	29.04	29.48
3	23.54	21.56	22.55
4	24.20	21.56	22.88
5	23.98	22.00	22.99
6	19.58	.....	.....
7	20.46	19.80	20.13
8	21.34	22.66	22.00
9	24.86	21.56	23.21
10	22.66	26.84	24.75
11	28.82	21.56	25.19
12	27.50	18.48	22.99
13	24.86	21.12	22.99
14	25.08	17.38	21.23
15	19.14	17.38	18.26
16	23.10	25.96	24.53
17	19.36	22.66	21.01
18	22.00	22.22	22.11
19	16.50	25.52	21.01
20	20.46	23.32	21.89
21	19.36	25.96	22.66
22	18.48	24.20	21.34
23	17.16	19.36	18.26
24	19.36	23.76	21.56
25	17.82	18.48	18.15
26	18.70	25.52	22.11
27	18.26	18.26	18.26

TABLE VI

MILLIGRAMS OF CO_2 PER KILOGRAM HOUR AT 0° C.
WINESAP APPLE

TIME AFTER PURCHASE IN DAYS	SAMPLE *c*	SAMPLE *d*	AVERAGE
1	56.98	15.28	36.13
3	26.84	17.82	22.33
5	12.54	14.30	13.42
7	18.48	14.30	16.39
9	16.50	25.96	21.23
11	20.46	26.40	23.43
13	15.40	24.20	19.80
15	29.48	15.18	22.33
17	22.22	22.22	22.22
19	25.08	20.68	22.83
21	25.52	29.92	22.72
23	9.90	14.96	12.43
25	12.76	12.10	12.43
27	32.12	16.72	24.42

TABLE VII

MILLIGRAMS OF CO_2 PER KILOGRAM HOUR AT 25° C.
OLDENBURG APPLE BRUISED

TIME AFTER BRUISING IN DAYS	SAMPLE *a*	SAMPLE *b*	AVERAGE	CHECK AVERAGE
1	72.16	60.06	66.11	51.70
2	60.72	60.50	60.61	75.24
3	63.36	63.36	63.36	64.02
4	64.68	62.04	63.36	64.90
5	69.96	57.64	63.80	62.70
6	69.30	64.46	66.88	55.88
7	70.62	60.06	65.34	53.68
8	60.06	52.58	56.32	53.90
9	70.84	48.40	59.12	51.26

Respiration determinations

The outstanding fact revealed by the data presented is the difference in respiratory behavior of Maiden Blush and Oldenburg apples, with respect to temperature changes. The rate for Oldenburg varied little with the temperature, while that for Maiden Blush showed a very large depression of respiration at 0° C. It should be noted that catalase activity is also low in the Oldenburg apple. The Maiden Blush specimens were somewhat immature, which may partially account for the low initial rate. Bruising had little effect on respiration, although it hastened decay. Unless injury is accompanied by greater freedom of gaseous exchange, it seems to exert only a minor influence upon the respiration rate. This result is similar to that secured by JOHNSTONE (**7, 8**) with the sweet potato. Maiden Blush mellows rapidly, while Oldenburg is a remarkable keeper for a summer apple. This would be expected in view of the respiration behavior here reported. The over-ripe specimens of Winesap gave little variation in rate due to temperature.

ENZYMES

CATALASE.—Oxidase and catalase activities are often reported as being correlated with respiration. It was decided, therefore, to make some determinations of the amount and local distribution of these enzymes in the fruit. Some idea of the localization of the enzymes is necessary in order to take samples intelligently. In the preliminary work catalase determinations varied greatly, depending

on the amount of skin and subepidermal tissue occurring in the sample. To obviate this variability, all samples for catalase determination were taken with a cork borer, going in through a cheek to the core of the apple. Precipitated calcium carbonate was used to neutralize the acidity of the tissue, and the dry weight was obtained from a duplicate sample taken from the same apple at the same time and about the same size as that used in the determination. Accuracy of the catalase determination requires the grinding up of all the skin.

TABLE VIII

LOCALIZATION OF CATALASE IN FRESH OLDENBURG APPLES; CC. OF O_2 LIBERATED BY FRESH EQUIVALENT 0.5 GM. DRY SAMPLE

TIME IN MINUTES	SKIN AND SUBEPIDERMAL TISSUE	FIRST CM. UNDER SKIN	SECOND CM. UNDER SKIN	CARPEL REGION
1	13.95	0.00	5.40	4.05
2	29.80	4.50	6.20	6.75
3	36.90	6.75	8.10	8.10
5	47.67	7.65	10.35	8.55

TABLE IX

CATALASE ACTIVITY IN FRESH OLDENBURG APPLES; CC. OF O_2 LIBERATED BY FRESH EQUIVALENT 0.5 GM. DRY SAMPLE

TIME IN MINUTES	SAMPLE *a*	SAMPLE *b*	AVERAGE
1	0.54	0.51	0.52
3	1.65	1.25	1.45
5	2.59	1.73	2.16
10	3.30	2.33	2.81

Hydrogen peroxide was used from a single fresh bottle secured from the Oakland Chemical Company, and all gas readings were reduced to 760 mm. pressure and 0° C. The apparatus used was a modification of that described by APPLEMAN (1). All determinations are for the equivalent of 0.5 gm. samples dry weight.

Tables VIII–XI present the results of catalase determinations. Table VIII shows the local distribution of catalase activity in an Oldenburg apple, while tables IX and XI provide a comparison of catalase in representative samples of Oldenburg and Winesap. Tables X and XI present the data for fruit (Winesap) stored at 0° and 25° C. respectively.

Catalase is much more active in the periphery of the apple (Oldenburg), and it is evident that the Oldenburg is low in catalase activity as compared with Winesap. This difference is not correlated with their respiration rates, for the Oldenburgs at 25° C. respired just about twice as rapidly as the Winesaps at the same temperature. Holding Winesaps at 25° C. brings about an increase in their catalase activity over those held at 0° C., as is seen from the data of tables X and XI. The significance of this difference is not known, since

TABLE X

CATALASE ACTIVITY IN FRESH WINESAP APPLES HELD AT 0° C.; CC. OF O_2 LIBERATED BY FRESH EQUIVALENT 0.5 GM. DRY SAMPLE

TIME IN MINUTES	SAMPLE *a*	SAMPLE *b*	SAMPLE *c*	SAMPLE *d*	AVERAGE
1	4.56	6.43	5.75	6.12	5.71
3	10.86	11.21	12.45	13.48	11.97
5	15.07	16.90	16.87	18.18	16.75
10	21.02	24.57	22.74	25.53	23.46

TABLE XI

CATALASE ACTIVITY IN FRESH WINESAP APPLES HELD AT 25° C.; CC. OF O_2 LIBERATED BY FRESH EQUIVALENT 0.5 GM. DRY SAMPLE

TIME IN MINUTES	SAMPLE *a*	SAMPLE *b*	SAMPLE *c*	SAMPLE *d*	AVERAGE
1	8.21	8.19	10.68	7.56	8.66
3	19.17	18.60	22.18	16.80	19.18
5	26.45	25.50	29.40	23.23	26.14
10	37.38	35.98	38.80	33.18	36.33

the respiration rates for this apple at 0° and 25° C. are practically the same.

OXIDASE.—Oxidase determinations, both qualitative and quantitative, were made in 1923 (4) and 1925. The simplified BUNZEL (2) apparatus, with pyrogallol or pyrocatechin as an absorbent, was used for the quantitative determinations. Color determinations, using an alcoholic solution of benzidine, as described by TUNMANN (16), were made for over fifty varieties of apples and a few samples of other pomaceous fruits. Many of these color determinations have been preserved in the form of photographic records. Tables XII and XIII show the quantitative distribution of oxidase in Winesap

and Yellow Transparent apples, and figs. 1 and 2 show the difference in local distribution of oxidase in these varieties.

Both the color and quantitative determinations indicated decreased oxidase activity when the fruit was hard ripe. This period was also characterized by less respiration than for ripe fruit. Red Astrachan apples showed this depression very clearly in the color tests (fig. 3). The decrease in oxidase activity at the hard ripe stage was quite marked in the periphery tissue of Yellow Transparent, and less marked in the core tissue of the same specimen.

TABLE XII

LOCALIZATION OF OXIDASE ACTIVITY IN WINESAP APPLES; CC. OF O_2 ABSORBED BY 5 CC. OF JUICE IN ONE HOUR

TISSUE	SAMPLE *a*	SAMPLE *b*	SAMPLE *c*	AVERAGE
Periphery	Trace	0.020	0.075	0.032
Core	0.290	0.143	0.259	0.231

TABLE XIII

LOCALIZATION OF OXIDASE ACTIVITY IN YELLOW TRANSPARENT APPLES; CC. OF OXYGEN ABSORBED BY 5 CC. OF JUICE IN ONE HOUR

TISSUE	IMMATURE	IMMATURE	HARD RIPE	NEARLY EATING RIPE	OVER-RIPE	AVERAGE
Periphery	0.090	0.080	0.000	0.023	0.066	0.052
Core	0.154	0.080	0.046	0.161	0.110	0.110

Winesap apples purchased on the market in July showed a higher oxidase activity than fresh picked specimens of Yellow Transparent. It is quite possible that the amount of acidity per variety influences this determination. Preliminary tests on sweet apples (low in acidity) indicate this.

The localization tests are even more interesting than the amount of oxidase activity per variety. Many varieties tend to have oxidase activity localized near the core. Varieties like Maiden Blush often show this localization in the periphery of the fruit (fig. 4). This suggested that localization of oxidase activity might be influenced by gas exchange. If such an exchange took place through the core, it ought to be possible, in time, to influence oxidase activity by sealing the

calyx tube in the hard ripe stage. Specimens of McIntosh, Delicious, Baldwin, and Spy treated in this way gave a negative test for oxidase activity in the core region after about two months at living room temperatures.

SHAW (14) found that northern grown specimens of Ben Davis were more conical than southern. Long shaped, conical specimens of Ben Davis having considerable flesh between the core and calyx tube showed oxidase activity in the periphery of the fruit. This suggests a climatic influence on oxidase activity in varieties of apples.

LOCALIZATION OF IRON

Iron is essential for chlorophyll development, and may be directly connected with the respiratory process. WARBURG (17) considers iron the only respiration catalyst of plant and animal cells. It was considered worth while, therefore, to secure some information on the localization of this element. Potassium ferrocyanide color tests were made on ashed samples of parts of the fruit. These qualitative tests indicated some localization of iron in the periphery.

TABLE XIV

LOCALIZATION OF IRON IN WINESAP; PERCENTAGE OF FRESH WEIGHT

TISSUE	SAMPLE *a*	SAMPLE *b*	AVERAGE
Periphery	0.00427	0.00592	0.00509
Flesh	0.00126	0.00152	0.00139

Quantitative determinations for iron were then made on flesh and periphery samples. These were taken by scraping with a glass knife, and all determinations were made in duplicate. The blanks indicated a very slight trace of iron in the reagents. The Neumann method, titrating with sodium thiosulphate, was used. Table XIV presents the results of these determinations for the Winesap variety.

Iron and catalase activity, therefore, are localized in the same general region of the Winesap.

Summary

1. Apple varieties vary greatly in respiration rate, and under some conditions show very little temperature response.

2. Respiration determinations give very little indication of ripening changes among varieties of apples after picking.

3. Oldenburg and Winesap apples respire less rapidly than Maiden Blush, other things being equal.

4. Skin and subepidermal tissues have comparatively high catalase activity.

5. Respiration rate and catalase activity are not closely correlated among apple varieties.

6. Oldenburg apples have low catalase activity compared with Winesap, together with a higher respiration rate.

7. Oxidase activity tends to decrease in hard ripe apples (Yellow Transparent and Red Astrachan).

8. Many apple varieties show greater oxidase activity near the core. Such varieties normally have a gas exchange through the calyx tube and core.

The writer desires to acknowledge his indebtedness to Professor C. A. SHULL and Dr. S. V. EATON of the University of Chicago for their kindly interest and many helpful suggestions through the course of the experiment.

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EXPLANATION OF PLATE XI

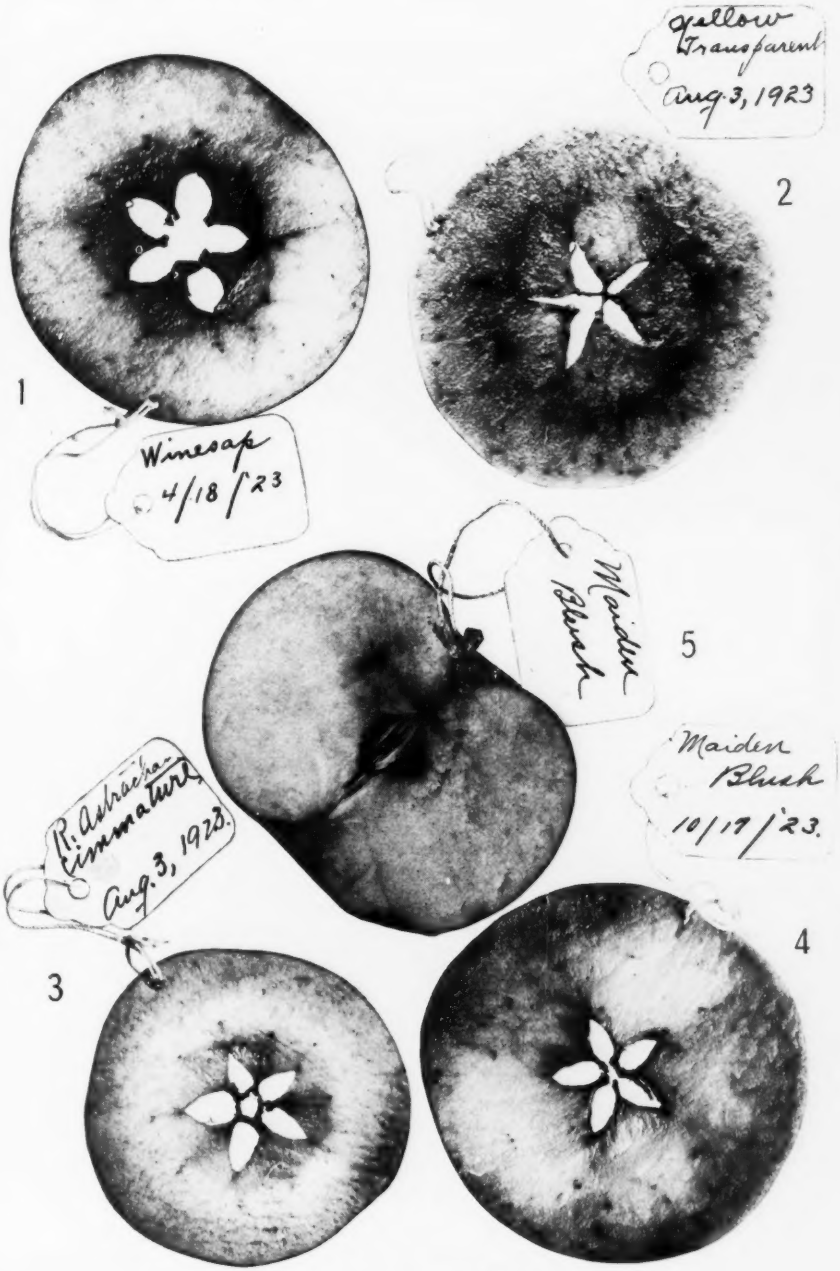
FIG. 1.—Oxidase activity in Winesap apples: dark color indicates oxidase activity; compare with table XII.

FIG. 2.—Benzidine oxidase activity test for Yellow Transparent apples; compare with table XIII.

FIG. 3.—Hard ripe Red Astrachan apples giving very small test for oxidase ripe specimens show considerable oxidase activity throughout the apple.

FIG. 4.—Oxidase activity in Maiden Blush apples; Westfield and northern grown Ben Davis apples show similar localization of oxidase activity.

FIG. 5.—Specimen of Maiden Blush showing flesh between calyx tube and core cavities.



DRAIN on ENZYMES OF APPLES

SURFACE FORCES OF SOILS WITHIN THE RANGE OF HYGROSCOPIC MOISTURE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 353

H. S. WOLFE

(WITH ONE FIGURE)

Introduction

The past decade has seen numerous and important advances in our knowledge of soil moisture, and in our recognition of the importance of the part played by the colloidal fraction of the soil in determining its moisture retaining properties. The conclusions which were reached by BOUYOUCOS (**4, 5**) as a result of the application to soil moisture problems of the dilatometer method employed with inorganic hydrogels by FOOTE and SAXTON (**9, 10**), have been particularly stimulating to research. His theory of the different physical states of the water present in the soil as soil moisture was at once challenged by various workers. KEEN (**14**), PARKER (**16**), and SHULL (**21**) have all examined this hypothesis, compared the data on which it was founded with their own data on a different angle of the problem, and concluded that there is no break or abrupt change in the physical condition of the soil moisture.

The fundamental error in the hypothesis of BOUYOUCOS seems to have been in assuming the trustworthiness of the dilatometer method. The work of VANZETTI (**25**) and LEHNER (**15**) has shown that this method does not give a reliable measure of the combined water in inorganic hydrogels, and consequently can hardly give an indication of the nature of the water present as soil moisture in soils, as PARKER has pointed out. THOMAS (**23**) has brought forward strong evidence that even with a relative humidity of only 1 per cent, the soil particles are covered with a film of water at least one molecule in thickness. The studies of VAN BEMMELEN (**24**), ZSIGMONDY (**26**), VANZETTI, and LEHNER on hydrogels; and of PATTEN and GALLAGHER (**17**), CAMERON and GALLAGHER (**7**), KEEN (**13**), SHULL (**20**), and PARKER on soils have all indicated that the water

present is all in the same physical state, but that it is held with great forces as its amount becomes small, forces so great, as PARKER indicates, that it cannot be frozen even at $-78°$ C.

In his résumé of the problem of soil moisture classification, PARKER has discussed the four methods by which these forces may be estimated relative to different moisture contents, and has shown the striking similarity in the curves obtained by these different methods. They are: (1) rate of evaporation, (2) freezing point depression at different moisture contents, (3) vapor pressure during dehydration, and (4) equilibrium relations of the soil with seeds of known water absorbing power. Of these methods, the second and fourth are by far the more sensitive, he says, and give curves of greater accuracy; but only the last method gives direct estimations of the values of the forces operating.

The only work done with this soil-seed equilibrium method has been that by SHULL (**20**), who devised it. Preliminary investigations had seemed to show that the graph which he presented for Oswego silt loam indicated too high a value for the forces at the point of maximum curvature. In view of the importance of this method of determining the forces with which soil moisture is retained, and of the questionable accuracy of the only published curve, therefore, it has seemed wise to reinvestigate more carefully the portion of the curve lying within the range of hygroscopic moisture, as this region includes all the debatable portion of the curve.

Methods and materials

The method and even the same apparatus described previously (**20**) were employed in this research, with certain modifications as noted. The rotator apparatus has been described and illustrated, so that no further reference to it is necessary here. Instead, however, of attempting to bring soils to an arbitrary water content by mixing the dry soil with water, air-dry soils were exposed for various periods of time in saturation chambers of the type devised by HILGARD (**12**) for studying the hygroscopic coefficient of soils. Since at least 24 hours are required for even a very thin layer of soil to reach its approximate maximum hygroscopic moisture content, it was conceived that by exposing the soil in layers of uniform thickness for

periods of time increasing by hourly increments from one up to 30 hours, a series of hygroscopic moisture contents would be obtained covering the desired range and with no large gaps in the line. Accordingly this was the procedure followed.

The soil was transferred as rapidly as possible from the saturation chamber to the rotator bottle, a known weight of air-dry seeds of *Xanthium* added, the soil shaken thoroughly to distribute the seeds well throughout it, and the bottle put into the rotator for two weeks. At the end of this time the bottles were opened, a few grams of soil rapidly transferred to weighing bottles, the seeds brushed free of dirt and put into weighing bottles also, and both soil and seeds weighed as soon as possible. From the increase in the weight of the seeds, the equivalent osmotic pressure of the soil "back-pull" was determined by inspection of a curve constructed from data previously given (**20**). From these data the hygroscopic moisture of the soil in equilibrium with the seeds was determined by drying the soil sample to constant weight in a 105° C. oven, and expressing the loss in weight as percentage of the oven-dry weight. The two sets of values thus obtained (equivalent osmotic pressure in atmospheres and hygroscopic moisture percentage) were plotted against each other to make more evident their relationship.

The seeds employed were from the laboratory stock of *Xanthium pennsylvanicum* Wallr. fruit, gathered from moderately pure line plants grown in the garden. Only the lower seeds were used, in order that the results might be based on as few non-uniform factors as possible, although such experiments as have been done with the upper seeds indicate no difference in their behavior in this respect from the lower seeds.

Two heavy loam soils were selected, following preliminary trials of a number of different soils to determine which were best suited for further experimentation, because they were readily brushed free from the seeds and because they resembled the soil used previously in this work. One came from a garden in Homewood, Illinois, and is designated "garden loam," while the other was one of a series of soils used in laboratory work, and is called simply "heavy loam." The following physical and chemical properties of these soils were determined and are compared in table I with the same properties

of the Oswego loam used by SHULL. All these are percentages of the absolute dry weight. The wilting coefficients and the moisture equivalents were calculated from the hygroscopic coefficient in the

TABLE I

TYPE OF SOIL	PERCENTAGE HYGROSCOPIC MOISTURE	PERCENTAGE HYGROSCOPIC COEFFICIENT	PERCENTAGE WILTING COEFFICIENT	PERCENTAGE MOISTURE EQUIVALENT	PERCENTAGE LOSS ON IGNITION
Oswego loam . . .	5.5	13.0	19.1	35.2	.....
Garden loam . . .	2.6	11.0	16.2	29.6	9
Heavy loam	3.9	14.0	20.6	37.8	11

TABLE II

HOURS IN SATURATION CHAMBER	HYGROSCOPIC MOISTURE OF SOIL IN PERCENTAGE OF OVEN-DRY WEIGHT		MOISTURE INTAKE OF SEEDS IN PERCENTAGE OF AIR-DRY WEIGHT		EQUIVALENT OSMOTIC PRESSURE IN ATMOSPHERES	
	Heavy loam	Garden loam	Heavy loam	Garden loam	Heavy loam	Garden loam
1	4.12	4.10	3.21	3.70	580	540
2	5.00	4.32	4.20	4.07	490	500
3	5.24	4.70	4.43	6.05	465	360
4	5.40	4.85	5.35	6.32	410	335
5	5.95	5.15	6.67	8.80	350	220
6	6.22	5.51	7.58	10.10	270	155
7	6.30	.....	7.96	.....	255	.....
8	6.84	5.55	10.69	11.73	177	130
9	7.00	.....	10.75	.....	165	.....
10	7.56	5.98	11.80	14.60	130	97
12	7.83	6.15	12.80	15.27	116	91
14	8.20	6.41	14.25	17.21	110	78
18	8.72	6.50	15.16	(20.97)	92	(61)
20	8.85	6.57	18.93	18.94	70	70
22	9.15	6.76	19.52	19.74	67	66
24	9.30	7.00	20.47	20.59	63	63
26	.....	7.60	.....	22.95	.....	54
28	.....	7.73	.....	24.05	.....	50
30	10.33	8.01	22.53	26.17	54	43
72	13.43	10.25	33.15	33.60	26	26

last two soils, while the process was reversed for the first one, the formulas of BRIGGS and SHANTZ (6) being employed.

Experimental results

LOAM SOILS AT VARIOUS MOISTURE CONTENTS.—The results of the experiments with heavy loam and garden loam are presented in

table II, while fig. 1 gives the curve obtained by plotting soil moisture against surface forces for these two soils and for that investigated previously.

The greatest care was taken to secure uniformity in the handling of the material, and to reduce sources of error to a minimum. ALWAY, KLINE, and MCDOLE (2) have pointed out the error due to

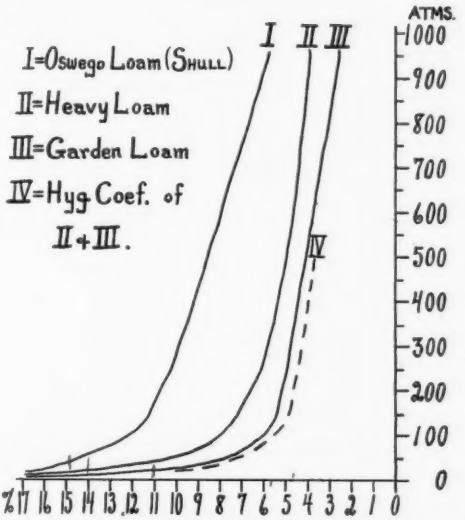


FIG. 1.—Curves showing relation of soil moisture content to soil surface forces: ordinates, surface forces of soil in atmospheres; abscissae for curves I, II, and III, moisture content of soil in percentage of absolute dry weight; for curve IV, moisture content of soil as percentage of hygroscopic coefficient (multiply figures by 10).

the loss of moisture while transferring soil from the saturation chamber to the weighing bottle, and compare the values for soils exposed during transfer for 30 and for 150 seconds. In these experiments the soil was exposed for less than 10 seconds. About 5 minutes were required for the cleaning of the seeds of each set, and they were partially exposed to the air during this period, but this has been found much too short a period to affect the resulting moisture content significantly.

Six samples of each soil constituted a series, the capacity of the rotator, and the same soil was air-dried for use in the next series. THOMAS (23) has pointed out that drying a soil previous to exposure increases its vapor pressure later for a given moisture content, but this is true only when the soil has been dried considerably below air-dry.

SOILS AT THE HYGROSCOPIC COEFFICIENT.—Several different soils were brought to the hygroscopic coefficient by three days' exposure in the saturation chamber. Table III presents the results obtained when the surface forces at this point were determined. For the

TABLE III

TYPE OF SOIL	MOISTURE INTAKE OF SEEDS IN PERCENTAGE OF AIR-DRY WEIGHT	EQUIVALENT OSMOTIC PRESSURE IN ATMOSPHERES
Sandy loam	31	30
Silty loam	34	25
Heavy loam	33	27
Garden loam	33	27

sandy loam the value given is the average of three determinations, and for the other soils it is the average of two determinations.

Since there is a slight loss of moisture during the experiment, so that the soils at its close are a little below the hygroscopic coefficient when in equilibrium with the seeds, the osmotic pressure equivalents are slightly too large. At the hygroscopic coefficient the value becomes about 25 atmospheres, when the correction for the moisture loss is made. Since the important value is the moisture content at the close of the experiment, that is, that when the seeds are in equilibrium with the soil, it is manifestly immaterial that the soil should not have exactly the same moisture content at the close as at the beginning. The loss is not due to any leakage of the apparatus, but to the length of time that the soil must be exposed in the process of getting it into the rotator bottle, and to loss of moisture within the bottle to saturate or bring into equilibrium with it the air in the bottle. The part actually absorbed by the seeds is negligible.

Discussion

The nature of the forces at work in the establishment of equilibrium between seeds and soil has been elucidated by SHULL (20), so that it is not necessary to consider that phase of the problem here. These data, however, make it clear that the hygroscopic coefficient represents a rather definite equilibrium point in the system soil particle-water vapor, for in these soils of different hygroscopic coefficient the force with which the soil particles hold back water is very constantly about 25 atmospheres.

This has been suggested previously by SHULL (21) on the basis of unpublished data, and is in line with what he has shown (20) to be the case with regard to the wilting coefficient, at which point a wide variety of soils were all found to hold back moisture with a force of 3–4 atmospheres. Incidentally it may be noted that when the surface forces at the wilting coefficient are calculated for the soils used in this experiment by extrapolation of the curves, the values agree closely with the preceding. The Oswego loam was shown by SHULL as having a back-pull of about 75 atmospheres at its hygroscopic coefficient, however, if this point may be calculated by the BRIGGS-SHANTZ formula (hygroscopic coefficient = moisture equivalent × 0.37). Using this equation the desired value is obtained as 13 per cent. Since neither SHULL nor the authorities he cites for the soil, CARTER and SMITH (8), make any mention of its hygroscopic coefficient, we cannot be sure that this value is correct, for ALWAY and RUSSELL (3) have shown that these formulas of BRIGGS and SHANTZ are not valid for all soils. They consider, however, that in general the mathematical derivation of the hygroscopic coefficient from an empirically determined moisture equivalent is more accurate than similar derivation from the wilting coefficient, and the figures furnished by BRIGGS for the Oswego loam (20) make it evident that he actually determined the moisture equivalent and derived the wilting coefficient from it, so that we are rather safe in deriving the hygroscopic coefficient similarly. It is felt that the abnormally high osmotic pressure equivalent found by SHULL for this soil at its hygroscopic coefficient is to be explained by his imperfect method for obtaining desired moisture contents. Soil mixed with water undoubtedly has its particles much less uniformly coated with

moisture films than when it is exposed in thin layers to a moisture laden atmosphere.

It has been noted that when the curves for all three of the soils in fig. 1 are prolonged to the point of the wilting coefficient, they all agree at about 4 atmospheres for the soil back-pull at this point. Now THOMAS (22) observed that the wilting point was reached when the vapor pressure of the soil had been lowered 0.4 mm. of mercury from its value at maximum air saturation. This he found to be equal to an osmotic pressure of 26 atmospheres in solution at 25° C., and so the soil should be retaining moisture with that force at the wilting coefficient. This is not at all in agreement with the numerous direct determinations, both of the actual force at the wilting coefficient, and of the moisture content when a force of 26 atmospheres is being exerted.

PURI, CROWTHER, and KEEN (18) call attention to the fact that at about 50 per cent relative humidity there is a minimum change in the moisture content of the soil for a given change in relative humidity. They suggest, therefore, that there may be thus derived a value which will express in a single soil constant as definite a characterization of the soil as any other single one. Air-dry soil has about 50 per cent relative humidity, and would thus be the basis of evaluation of the soil constant. In the present investigation the air-dry moisture content showed a much less constant relation to the surface forces than did the hygroscopic coefficient.

Very interesting results were obtained by comparing the hygroscopic moisture of the two soils used in this experiment, expressed as percentage of the hygroscopic coefficient, at certain definite osmotic pressure equivalents. From the remarkable uniformity of these values for these two soils, as shown in table IV, we are led to question whether the hygroscopic coefficient may not possess more significance as a soil constant than has lately been conceded to it.

Curve IV in fig. 1 shows graphically these relationships between the surface forces of the soil and its moisture content expressed in terms of the hygroscopic coefficient, and the striking similarity in its form to that of the usual soil moisture curves beside it. The force with which soil particles retain moisture appears to be a function of the hygroscopic coefficient, and is equal for different soils

when they contain the same percentage of their maximum hygroscopic moisture capacity.

In accordance with our modern concepts of the part played by colloidal matter in soil surface forces, we should expect that sand, which is deficient in colloid content, would fail to follow the same laws as soils, and so it proves. KEEN (13) found evaporation from sand to proceed quite differently from that from soil, following instead the usual laws of physical diffusion. SHULL (20) found coarse sand to give values of back-pull at the wilting coefficient considerably divergent from those uniformly obtained for the other seven

TABLE IV

OSMOTIC PRESSURE EQUIVALENT (ATMOSPHERES)	SOIL MOISTURE CONTENT AS PERCENTAGE OF HYGROSCOPIC COEFFICIENT	
	Heavy loam	Garden loam
25	100	100
50	73	71
75	62	60
100	57	55
150	51	50
200	47	46
250	45	45
500	36	38

different soils examined. The present experiments have shown that at the hygroscopic coefficient a coarse sand retains moisture with an equivalent osmotic pressure much below that for soils, and that there is no constant relationship between those forces and the hygroscopic coefficient. If this equilibrium point may be calculated for the sand used in the present work, it was there found to correspond to a surface force of only 10 atmospheres, and for different sands this value differs also. We are dealing evidently with quite different factors from those which operate in soils. It is of some interest to note that both in the present and in previous work, sand at about three times its air-dry moisture content showed about 30 atmospheres of surface force, but in the former case this was at 10 per cent of the hygroscopic coefficient, and in the latter case at about 50 per cent of that moisture content.

The application of this determination of the soil surface forces at the hygroscopic coefficient may be made through the work of ALWAY (1), who noted that many desert perennials could live in soils which were below this moisture content. This means that they were able to extract water from the soils against a back-pull of more than 25 atmospheres of force. Desert plants are well able to produce within their root cells osmotic forces far greater than this, as HARRIS, LAWRENCE and GORTNER (11) have demonstrated, and, furthermore, are able to withhold water from transpiration by the development in their leaves of very powerful hydrophile colloid pentosans and mucilages, as various workers at the Desert Laboratory have pointed out (21).

A number of improvements in technique have been suggested by various workers since this work was begun, and more accurate methods are now available which should give even more certain data. ROBINSON (19) has pointed out the advantages of 2 per cent H_2SO_4 for use in obtaining the hygroscopic coefficient, since it gives values approaching those obtained over water, and yet permits the establishment of a real equilibrium such as is never obtainable with HILGARD'S method. THOMAS and PURI, CROWTHER and KEEN have added greatly to our knowledge of methods for soil vapor pressure determination. They have established the value of the vacuum desiccator, using concentrated H_2SO_4 as dehydrating agent, for the drying of soils to constant weight, and of the same apparatus with H_2SO_4–H_2O mixtures of known vapor pressure for producing varied soil moisture contents. Drying over H_2SO_4 gives more constant and lower absolute dry weights than does drying in the 105° C. oven, and avoids the changes in colloidal structure incident to heating. The difficulties due to the hysteresis of the colloidal portion of the soil in its water relationships are just as real with air-dry soil as with soil saturated with moisture vapor. The value of the constant suggested by KEEN and his co-workers lies in the fact that moisture content varies little over a considerable range of relative humidity of about 50 per cent relative humidity, and so a definite soil moisture content is readily obtained with some certainty. But with their method of producing desired relative humidities, it is possible to obtain one of 95 per cent, which is about what can be obtained in the usual HILGARD method, with equal precision, and to produce very uniform

hygroscopic coefficients. Until the moisture content at 50 per cent relative humidity is shown to have more constant relation to the empirically determined soil surfaces, as further work may show it to have, the hygroscopic coefficient remains our most useful concept as a soil characterizer.

Finally it may be noted briefly that the smoothness of the curves given in fig. 1 for the soil surface forces indicates once more the impossibility of considering that water is present in more than one physical state in soils normally.

Summary

1. Various soils are shown to hold moisture when at their hygroscopic coefficient with a force uniformly equivalent to about 25 atmospheres, as measured by the seed-soil equilibrium method of SHULL.

2. For at least the two loam soils investigated, the surface soil forces within the range of hygroscopic moisture are shown to be constant for any given soil moisture content, expressed as percentage of the maximum hygroscopic moisture capacity of the soil, that is, to be functions of the hygroscopic coefficient.

3. Sand does not follow the same laws in regard to moisture holding forces as do soils.

4. The curve for soil forces given previously is shown to indicate too high values for the force with which water is retained through that portion of the hygroscopic moisture range near saturation with water vapor.

The writer desires to express his thanks to Professor CHARLES A. SHULL for suggesting this problem, and for constant aid and encouragement throughout the process of its solution.

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OEDOGONIUM NEBRASKENSIS, SP. NOV.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 354

HIRO OHASHI

(WITH TWENTY FIGURES)

In April 1919, Dr. ELDA WALKER found an *Oedogonium* which seemed to be new. The late Professor COLLINS of Tufts College made a preliminary examination of the material and thought the species might be new, but he died before he had an opportunity to make a thorough study. I wish to express my thanks to Dr. WALKER, who kindly turned the material over to me.

I am indebted to Professor CHARLES J. CHAMBERLAIN for criticism and suggestions; and also to Professor E. N. TRANSEAU and Dr. H. TIFFANY, who examined the material and corrected my taxonomic description of the new species.

Material and methods

The following description of the location where the material was collected was supplied by Dr. WALKER:

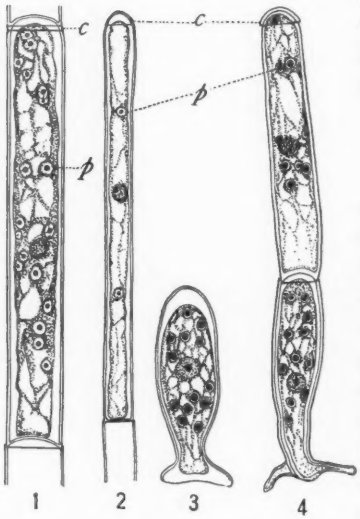
> The material was collected April 22, 1919, in the small ponds along a branch railroad line running into the State Fair grounds at Lincoln, Nebraska. The pond from which most of it came was about 50 ft. long and 10 ft. wide. The deepest parts measured hardly a foot. It was a few rods east of the northeast corner of the Fair grounds enclosure. The ponds were full of grasses and prairie plants as they are dry most of the year and seldom retain water any length of time. That was a wet spring and the water stood in the ponds for some time.

The material was killed, fixed, and preserved in a chrom-alum solution: water 100 cc.; chrom alum-2 gm.; formalin 1 cc. It was washed with running water and stained with iron-alum haematoxylin, and also with Magdala red and anilin blue; then it was mounted in Venetian turpentine.

Morphology

The aquatic filament is very small, about 1.5 inches in length, adhering to the surface of grass blades or stems of other plants, giving them a loose velvet-like appearance. It is dioecious, anthe-

ridia and oogonia occurring on separate filaments. The female plant consists of a single series of uninucleate cells, numbering 20–40 (occasionally about 50), including vegetative cells, androsporangia, oogonia, and suffultory cells. The prevailing number is 21–25. The male filament or dwarf male is very much smaller than the female, consisting of just a few cells; so the species is nanandrous.



FIGS. 1–4.—Fig. 1, vegetative cell with reticulate chromatophore, nucleus, and pyrenoids; fig. 2, terminal cell with cap; figs. 3, 4, sporelings showing basal cell with holdfast; ×780.*

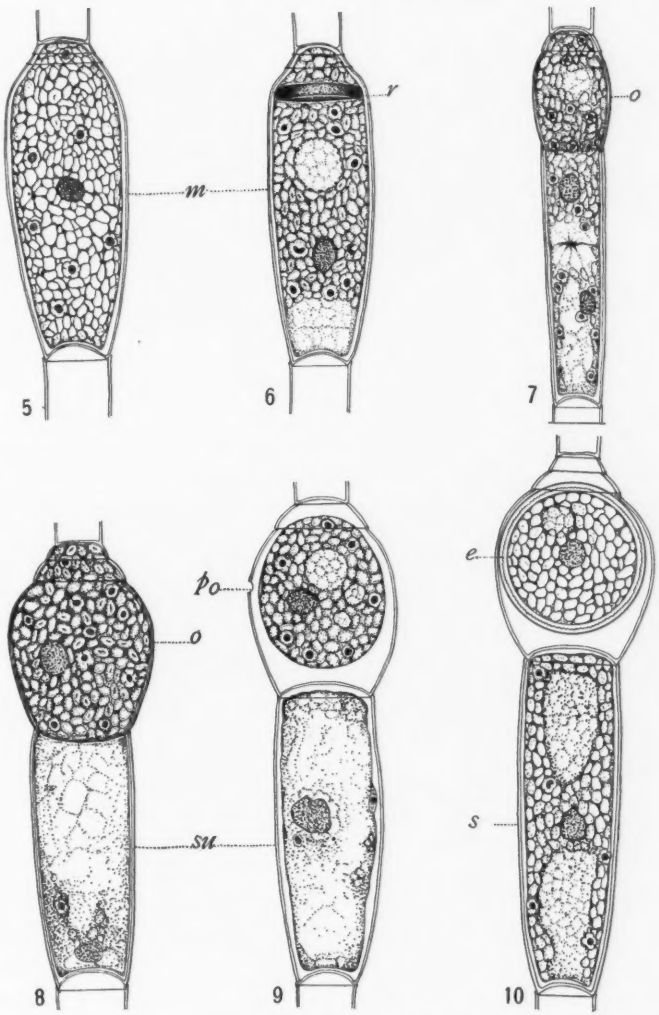
*The following abbreviations are used in all the figures: *a*, androsporangia; *an*, antheridium; *c*, cap; *e*, oospore; *m*, mother cell; *o*, oogonium; *p*, pyrenoid; *po*, pore; *r*, ring; *s*, sperm; *su*, suffultory cell.

VEGETATIVE CELLS

The vegetative cells are cylindrical, containing peripheral reticulate chromatophores, along which many pyrenoids are scattered (fig. 1). Each pyrenoid is usually surrounded by starch grains. The vegetative cells are slightly capitellate, the terminal cell being attenuate and obtuse (fig. 2), while the basal cell is elongated and has a well developed holdfast (fig. 4). The zoospore germinates into a basal cell (fig. 3), which gives rise to a whole filament by the special type of cell division characteristic of *Oedogonium*, forming at the upper end of the cell a circular ring which makes a new cell wall for the daughter cell. As the result of this kind of cell division, many caps are formed at the upper extremity of the cell.

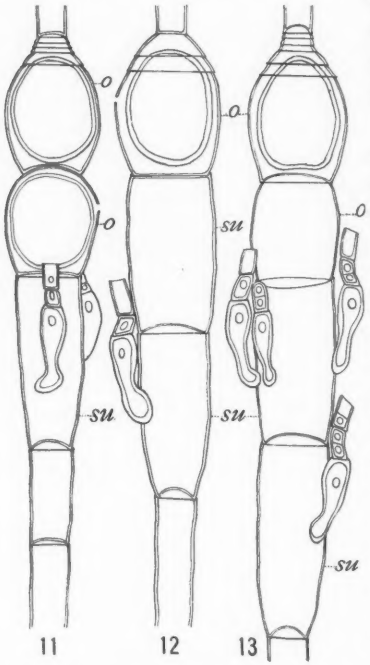
REPRODUCTIVE CELLS

OOGONIUM.—The formation of the oogonium is started by swellings of the usual vegetative cell which is called the mother cell (fig. 5), and is followed by the appearance of a circular ring at the top (fig. 6), and then by a division of the mother cell (fig. 7). By



FIGS. 5–10.—FIG. 5, mother cell of oogonium and suffultory cell; fig. 6, ring in mother cell; fig. 7, cell division of mother cell; fig. 8, young oogonium: fig. 9, formation of pore and oosphere; fig. 10, oospore with three layers of cell wall; ×780.

this division the upper one of the two daughter cells grows into an oogonium, while the lower one is a suffultory cell. As soon as the wall of the oogonium is formed by the rupture of the circular ring, the greater part of the contents of the mother cell moves into the oogonium, leaving large vacuoles behind. Then a cross wall is formed and the oogonium begins to swell (fig. 8.)



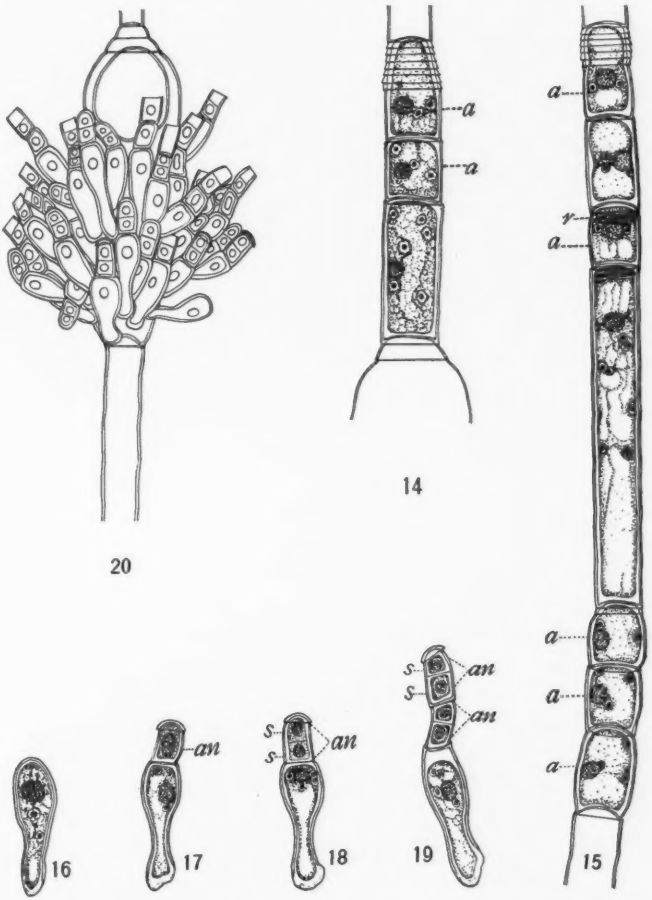
FIGS. 11–13.—Fig. 11, two oogonia on one suffultory cell; fig. 12, one oogonium on two suffultory cells; fig. 13, two oogonia on two suffultory cells; ×480.

After attaining a certain size, a pore is formed at the supramedian part of the oogonium wall, and the contents of the oogonium begin to constrict and round off, forming a single oosphere (fig. 9). Then a sperm from the dwarf male enters the oosphere through the pore and fertilization takes place. After fertilization the wall of the oospore is formed, with three layers, the outer and inner layers which are smooth, and a median layer which is punctate (fig. 10).

The number of oogonia is usually one in a filament, and it rests upon a single suffultory cell (fig. 10), but it is not rare to find two oogonia on a single suffultory cell (fig. 11), or on double suffultory cells (fig. 13), and sometimes there is a single oogonium on two suffultory cells (fig. 12). Occasionally several oogonia arise on the same filament, separated from one another or occurring in immediate succession. The oogonia are flask-shaped or oviform; the oospores, not quite filling the oogonia, are mostly subspherical or oviform (figs. 11–13), rarely quite spherical (fig. 10).

ANTHERIDIUM.—The dwarf male is produced by germination of the androspore which came from the androsporangium. The andro-

sporangia, occurring in the female filament at a short distance above the oogonium, are much smaller than the vegetative cells (fig. 14).



FIGS. 14–20.—Figs. 14, 15, androsporangia; figs. 16–19, development of dwarf male; ×780; fig. 20, suffultory cell surrounded by more than thirty dwarf males; ×480.

They are either single or double, sometimes three or four in a series (fig. 15). When androsporangia are developed on the female plant

the filament is called "gynandrosporous." The motile ciliated androspores escape from the androsporangia, and after swimming around for a while, attach themselves to the suffultory cell and begin to develop into dwarf males.

The androspore forms first a basal vegetative cell, the so-called stipe (fig. 16). The stipe then gives rise to one or two, rarely three antheridia by typical *Oedogonium* cell division; this type of formation of the antheridia is named "antheridium exterior," one being placed above the other (figs. 17–19). It is very common to find

TABLE I

Name of cell		O. concatenatum f. Hutchinsioe	O. nebraskensis (μ)	Remarks
Vegetative cell	Diameter	26–35 μ	20–35	Very close
	Length	3– 6 diam. long.	57–236	Some difference
Suffultory cell	Diameter	37–50 μ	41–58	Close
	Length	1¾– 4 diam. long.	93–130	Some difference
Oogonium	Diameter	52–75 μ	60–67	Rather close
	Length	67–95 μ	70–79	Slight difference
Oospore	Diameter	50–73 μ	53–64	Close
	Length	55–77 μ	60–76	Close

several dwarf males on a suffultory cell; however, in one case thirty dwarf males were seen surrounding a single suffultory cell (fig. 20).

Taxonomy

Measurements of all kinds of cells in *O. nebraskensis* are given in the technical description of the species. The new species is very close to *O. concatenatum* f. *Hutchinsioe* (Wittr.) Hirn, from which it differs in dimensions, in having a punctate spore wall rather "scrobiculate," and in having the pore supramedian instead of "superior." Table I compares the dimensions of some cells of both species.

As shown in table I, *O. nebraskensis* is different from *O. concatenatum* f. *Hutchinsioe* in the dimensions of the cells. The oogonium and the oospore of both species are not so dissimilar in their diameters and lengths. The diameters of the vegetative cell and of the

suffultory cell are also not so different, but the length of both cells in *O. nebraskensis* is somewhat different from that in the other species. Another characteristic of this species is the marking of the cell wall of the oospore, in which there are three spore coats, with the median coat punctate; while that of *O. concatenatum* f. *Hutchinsioe* is "scrobiculate." One more outstanding character of this *Oedogonium* is the position of the pore in the oogonium. This is supramedian instead of "superior," which is represented in the other species with which it is compared. The following is a technical description of the new species.

Oedogonium nebraskensis Ohashi, sp. nov.—Dioecious, nannandrous, gynandrosporous; oogonia single or 2–4, flask-shaped or oboviform, pore supramedian; oospore subspherical or oviform, not quite filling the oogonium; outer spore wall smooth, median wall punctate, inner wall smooth; suffultory cell swollen; androsporangia 1–4-celled; basal cell elongate; terminal cell attenuate obtuse; dwarf males curved on suffultory cell; antheridium exterior, 1–3-celled; vegetative cells slightly capitellate; whole filament 20–41 celled (occasionally about 50).

VEGETATIVE CELLS

	DIAMETER IN μ	LENGTH IN μ
Terminal cell	9–12	170–305
Median cell	20–27	57–160
Basal cell	22–35	79–230

REPRODUCTIVE CELLS

Suffultory cell	41–58	93–130
Oogonium	60–67	70– 79
Oosphere	53–64	60– 76
Androsporangium	20–25	22– 40
Stipe of dwarf male	12–19	47– 68
Antheridial cell	9–13	17– 20

Summary

1. It might be better to treat this material as a new variety of *O. concatenatum*, so far as the dimensions of the oogonium and oospore and the diameter of the vegetative and suffultory cells are concerned, because there is not enough difference in dimensions to establish a new species. We cannot overlook, however, the differ-

ence existing in the length of the vegetative cells and of the suffultory cells in both species.

2. The marking of the median spore coat of the oospore of *O. nebraskensis* is different from that of *O. concatenatum* f. *Hutchinsioe* in being "punctate," while the latter is "scrobiculate."

3. The most distinguishing character of this species is the position of the pore in the oogonium, in the supramedian position instead of the superior.

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GERMINATION OF SPORES AND EARLY STAGES IN DEVELOPMENT OF GAMETOPHYTE OF MARCHANTIA POLYMORPHA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 355

SISTER MARY ELLEN O'HANLON

(WITH PLATES XII–XV)

This study was undertaken to determine whether there is a definite apical cell which acts as the initial in the development of the gametophyte of *Marchantia polymorpha*, as has previously been stated. LEITGEB[1] states: "In reference to the already variously studied germination, one might only mention that with all the variety in the development of the first stages, there is always finally formed at the apex a two-faced apical cell." CAMPBELL[2] says: "In some of the thallose forms, e.g., Marchantiaceae, Anthocerotes, a single initial cell is not always to be recognized in the older thallus, but in these forms a single initial always appears to be present in the early stages."

Material

The spores for this investigation were collected by Miss CAROLINE WEBER, at Pelican Lake, Oneida County, Wisconsin, during the summers of 1923 and 1924. At that latitude (45° 30′ N), the season for spore dispersal begins during the latter part of July, and spores may be collected even later than September 15. At Sinsinawa Mound, Grant County, Wisconsin, about 3° farther south, the season for ripe spores begins in early July and collections may be made toward the end of August. Plants kept under greenhouse conditions shed their spores as early as May 15.

For study of the differentiation of elaters and sporogenous tissue, *Marchantia* plants were collected at Sinsinawa Mound. In the development of the intracapsular tissue of the sporophyte, all of the cells are fundamentally sporogeneous. Later certain cells divert and

[1] LEITGEB, H., Untersuchungen über die Lebermoose 6:121. 1881.

[2] CAMPBELL, D. H., Mosses and ferns. 2d ed. 1905 (p. 15).

are destined to metamorphose into elaters. The elaters are discernible in an incipient stage five generations before the spore mother cells appear; that is, the diverted cells are sisters to those sporogenous cells which further undergo five series of divisions before they reach the spore mother cell stage. It is estimated that in *M. polymorpha* there are 32 spore mother cells to each elater, therefore 128 spores to each elater.

Spores

The spores of *Marchantia* are exceedingly numerous, although very small. In order to calculate the spore output of a single head, the writer examined over 100 archegonial heads. The number of groups of archegones ranges from 6 to 10, the average and dominant number being 8. Fig. 1 shows that the number of archegones in each group is up to 16, cases having been observed in which there were 18 archegones in a single group.

Although most of the fertilized eggs sometimes undergo a series of cell divisions and sporophyte embryos begin development (fig. 2), it is not likely that more than three sporophytes of a single group, and those nearest the food supply, ever come to maturity (fig. 3). The number of spores in a single capsule is reckoned as not less than 300,000. With an average of 24 mature sporophytes to a head, the total spore output for a single head of *M. polymorpha* is more than 7,000,000 spores.

Methods

Of the various subtrates upon which germination was tried, such as soils, wood ashes, charcoal, liquid media, and porcelain plates kept moist with Knop's solution in glass chambers, the last was much the best. This method is superior not only for the growing facilities which it affords, but also for subsequent preparation of the sporelings, which in *Marchantia* are exceedingly small, due to the minuteness of the spores. Spore cultures tried out in the greenhouse were relatively unsuccessful because of the intrusions of algae. Satisfactory results were obtained with cultures grown indoors in east windows of a white walled room with north, south, and east exposures.

Spore germination

The spores of *Marchantia polymorpha* germinate readily upon being shed or shaken from the heads, and they are 100 per cent viable for about a year when kept in envelopes at room temperature. After a year's elapse, the spores rapidly lose their vitality, so much so that spores 14 months old were probably not more than 50 per cent viable; those 17 months old failed totally to germinate. Germination is almost immediate after the spores are sown, and ordinarily begins by a uniform enlargement accompanied by chlorophyll development, until the diameter of the germinating spore is about double that of a freshly shed spore (fig. 5 *a-b*). In the method of germination, and more especially in the subsequent development of the sporelings, however, there is considerable diversity of activities, as well as marked unevenness in the degree of advancement within the same length of time. These differences are influenced particularly by environmental conditions, but also by a certain capriciousness which seems to be a subjective quality of the sporelings themselves, as there is more or less variety in the forms which appear in a single culture. In most cases, a rhizoid appears after a uniform growth of the spore and simultaneous development of chloroplasts have occurred (fig. 4 *c*). Occasionally a second rhizoid appears early, even in the 2-celled stage, the spore giving rise to both (fig. 4 *g*). The second rhizoid from the spore cell may not appear, however, until the young gametophyte has advanced considerably (fig. 5 *g*).

Early stages in gametophyte development

The first cell divisions are usually in one plane only, until a row of cells is formed. The number of cells in the filament is increased under weak light conditions, and accordingly divisions in a second plane are retarded. In light of considerable intensity and long periodicity, divisions in the second plane occur most frequently when the filament is but three or four cells in length. Figs. 4 and 5 show the variety of forms which appear in these early stages. Thus cell divisions, alternating with periods of growth, continue rapidly. Then there comes a time, while the plant is still young and limited to probably less than a dozen cells, when a marginal row of cells appears in the apical region of each plant or branch of each plant, ac-

cording as branching occurs earlier, as it frequently does (fig. 6 *b–f*). This marginal row of embryonic cells is conspicuous throughout subsequent development, once it is established. The writer examined hundreds of young plants in all stages of germination and growth under a variety of environmental conditions. There were relatively few that showed anything like a wedge-shaped, or otherwise characteristically shaped cell to which any significance as to its potentiality might be attached. Occasionally, in very early stages, before the marginal row is established, there appears an end cell which challenges attention (fig. 6 *a*). There are many more sporelings, however, that manifest a marked individuality in their course of development, rather than anything like a regular procedure. LEITGEB shows only one figure to support his theory. This is not sufficiently convincing, and one is tempted to conclude that too few specimens were examined. Moreover, the technique available for his extensive investigations on the liverworts was primitive as compared with our present facilities.

In the activity of the embryonic row of cells at the margin of the young plant, differential growth brings about a characteristic notch in the apex (figs. 8–10). In this marginal row of cells, the sum of their outer walls must necessarily exceed in length the combined widths of their inner walls. It is plain, therefore, that the cells of the periphery, some of them at least, must incline to a wedge shape. It is impossible, however, to reconcile the position, form, and apparent function of any cell at the apex of a young thallus so as to designate it as *the* initial cell.

Dorsiventrality is established by the budding off of secondary rhizoids from the side in contact with the substrate. These rhizoids arise somewhat behind the apical notch, and in a line which later becomes the midrib of the young thallus (figs. 11, 12). Like all of the rhizoids which appear in the young gametophytes, these are of the plain walled type. The pegged rhizoids fail to appear until a later stage is reached. Coincident with this, the rise of mucilage cells at the apex insures the anchorage of the young thallus. At about this point too, certain cells, either in the peripheral row or in the outer areas of the plant, manifest themselves as storage organs of essential oils. These cells not only have a characteristic form, and a

diminished size as compared with the other cells of the thallus, but react with osmic acid to indicate their contents (figs. 12, 13).

From the foregoing premises, the conclusion seems to be well warranted that not only is there no single cell upon which the destiny of the plant depends, but that any cell which has retained its vitality is potent to initiate an individual course of action. This individual cell behavior is not alone to send out a rhizoid or to cause a differentiation of parts, but also actually to give rise to a branch which later becomes a distinct plant (figs. 6 *e*, 7 *c*, 9 *a*, 10 *a*, and 11). Just what part environment or various stimuli may have to play here in determining the course an active cell may take cannot be defined, but fundamentally the response must be due to inherent factors in the cells themselves.

Effects of light, temperature, and moisture

Experiments were tried with spore cultures in daylight prolonged with electric light or shortened by darkening, so that many gradations of light periodicity were devised, under conditions which, other than light, were identical. In the beginning, it was observed that the plants in light periods longer than 16 hours and up to 24 hours advanced more rapidly than those subjected to shorter light periods. This condition prevailed for about three weeks from the date the spores were sown, after which there was a decline in the growth of the sporelings subjected to the long or continuous light periods. At the same time there was a steady increase in the growth of the sporelings which were exposed to 9–16 hours of illumination. The optimum light periodicity for steady and persistent growth seems to be 13–15 hours. Spore cultures placed in weak light, with north exposure, germinated but seemed to produce algal-like forms (fig. 14).

On September 8 experiments were made with cultures, some set up indoors and others out-of-doors. The plants grown out-of-doors were probably a little slower at first, but after the germination stage they much surpassed those of the indoor cultures. This indicates that light of moderate intensity is better for germination, while light of higher intensity is more satisfactory later on. Again, sparse sowings are more successful than profuse sowings, probably more because of shading in the case of the latter than for any other reason.

Figs. 15 and 16 show samples of the indoor and out-of-door cultures respectively as they appeared on September 24, just 16 days after the spores were sown. Figs. 17 and 18 show samples of the same cultures on October 1. It is plain that the out-of-doors and therefore better light conditions are more favorable to the growth of the plant, and this in spite of the fact that higher temperature (which prevails indoors) is better for vegetative growth. This latter point was demonstrated by cultures which were transplanted to soils in flower pots and kept under greenhouse conditions all winter. Here the light intensity and periodicity were the same for plants, some at 10°–15° C. and others at 18°–22° C. Those kept at the higher temperature much exceeded the others in vegetative growth, but fruited much less abundantly than those in the cooler section of the greenhouse. Temperature, however, is less a factor in both the vegetative and fruiting functions than light, longer periodicity being essential[3] for the latter, and decidedly better for the former.

Spores sown in Knop's solution in Petri dishes begin germination as readily as those upon a solid substrate, but the course of procedure is different. The bizarre structures which obtain under such conditions are typified in fig. 19. When a liquid medium culture is kept in weak light, with north exposure, the unsuccessful attempts at thallus development are as shown in fig. 20. It is evident that *Marchantia*, although less capable of withstanding drought[4] than most of its relatives, is by no means inclined to a hydrophytic habit. On the other hand, the sporelings of *Conocephalum*, when sown on liquid media, continue development through a series of stages which are not unlike those which obtain when its sporelings are sown on a solid substrate. This is true, although the adult plants of *Conocephalum* are much more resistant to drought and appear to be much more strictly mesophytic. As a matter of fact, *Marchantia* is limited to a mesophytic habit throughout, and has a rather narrow range between what borders on drought on the one side and excessive moisture on the other.

[3] Certain observations recently made indicate that this is true only in the earlier stages in the development of the antheridia.

[4] The writer wishes to except the gemmae of *Marchantia*, concerning the rôle of which a later paper will appear.

Summary

1. Spores of *Marchantia polymorpha* are available in this general latitude, depending upon the climatic conditions, from early July to the middle of September.

2. Elaters of *Marchantia* are sister cells of sporogenous cells which undergo five divisions before the spore mother cells appear.

3. The ratio of the number of elaters and spores is 1:128.

4. The total number of spores in a single head is estimated to be about 7,000,000.

5. The spores are viable for about a year.

6. One (rarely two) primary rhizoid appears after the growth of the spore and chlorophyll development has occurred.

7. Under ordinarily good conditions for germination, cell divisions giving rise to a very short filament are followed by cell divisions in a second plane.

8. Branching of the young thalli is not uncommon and sometimes occurs at a very early stage.

9. A marginal row of meristematic cells is early established, and these, rather than a single apical cell, are active throughout subsequent development.

10. By differential growth of the cells in the marginal row, a notch appears in the apical region when the young gametophyte comprises 30–40 cells.

11. Dorsiventrality and anchorage are established by the budding off of rhizoids behind and in a line perpendicular to the apical notch, and by the rise of mucilage cells on the lower side of the apex.

12. Excepting during germination, when more moderate light seems better, 13–15 hours of light of good intensity is the optimum.

13. The optimum temperature conditions for vegetative growth are 18°–22° C., and the optimum temperature conditions for fruiting are 10°–15° C.

14. As a medium for successful germination and growth, a solid substrate is much better than a liquid one.

The writer wishes to express sincere gratitude to Dr. W. J. G. LAND, who suggested this investigation, and under whose direction

it was made, and to Mr. ROBERT G. GUTHRIE, who did all of the photographic work.

ROSARY COLLEGE
RIVER FOREST, ILL.

[*Accepted for publication October 11, 1925*]

EXPLANATION OF PLATES XII–XV

FIG. 1.—Transverse section of young head, showing numerous archegones in each group ; ×47.

FIG. 2.—Segment of transverse section of later stage of archegonial head, showing numerous young sporophytes in each group; ×30.

FIG. 3.—Group of three sporophytes; ×107.

FIG. 4.—Spore germination: *a*, freshly shed spore; *b–l*, various stages in germination and development of young gametophytes; ×150.

FIG. 5.—Typical young gametophytes.

FIG. 6.—More advanced stages of young gametophytes: *e*, young plant showing early branching; ×150.

FIG. 7.—Young gametophytes all showing marginal row of cells at apex; *c*, branching thallus; ×150.

FIG. 8.—Two slightly different forms at about the same stage of development: *a*, type of development where light is less intense; *b*, showing slight dip to form the apical notch; ×150.

FIG. 9.—Later stages in development of thallus: *a, young gametophyte* showing three apices; *b*, young thallus showing apical notch; ×150.

FIG. 10.—More advanced stages: *a, branching thallus*, ×150; *b, typical* unbranched thallus; ×100.

FIG. 11.—Photomicrograph of *branching thallus*; ×125.

FIG. 12.—Photomicrograph of thallus showing thickened apical notch, two budding cells behind apical notch, and storage cells of essential oils in periphery; ×175.

FIG. 13.—Detail of peripheral region of fig. 12, showing two storage cells; ×480.

FIG. 14.—Gametophytes grown in weak light.

FIG. 15.—Photomicrograph of young gametophytes grown indoors, September 8–24; ×40.

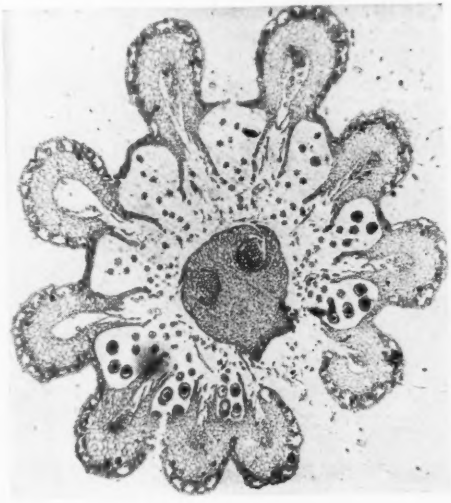
FIG. 16.—Young gametophytes grown out-of-doors, September 8–24; ×40.

FIG. 17.—Young gametophytes grown indoors, September 8–October 1; ×40.

FIG. 18.—Young gametophytes grown out-of-doors, September 8–October 1; ×40.

FIG. 19.—Sporelings grown on liquid medium: *a*, freshly shed spore; *b–f*, various stages of abnormal development; ×150.

FIG. 20.—Sporelings grown in weak light on liquid medium; ×100.



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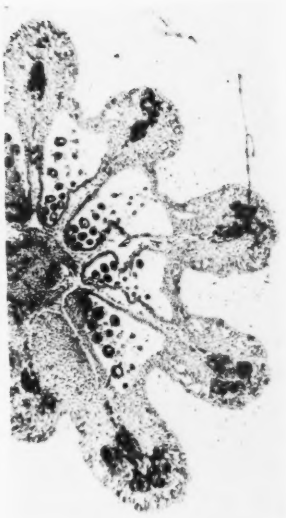

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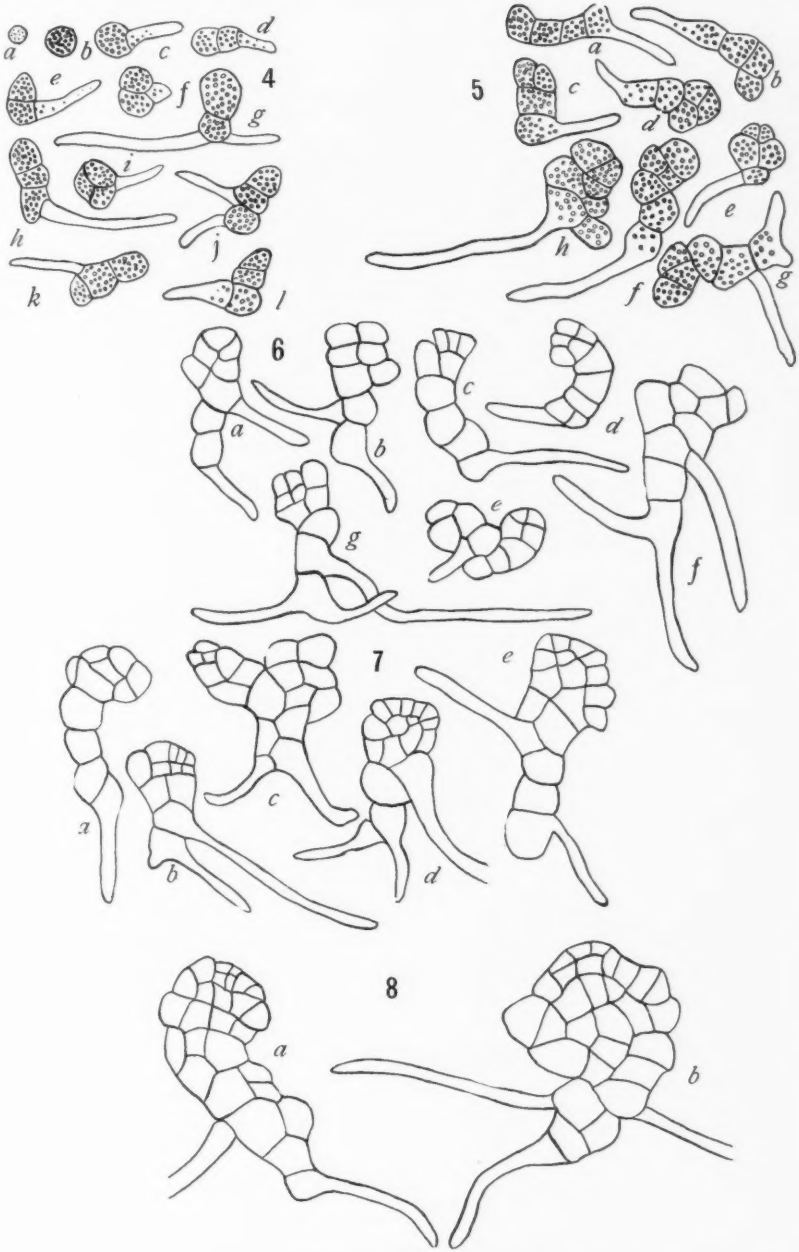
O'HANLON on MARCHANTIA



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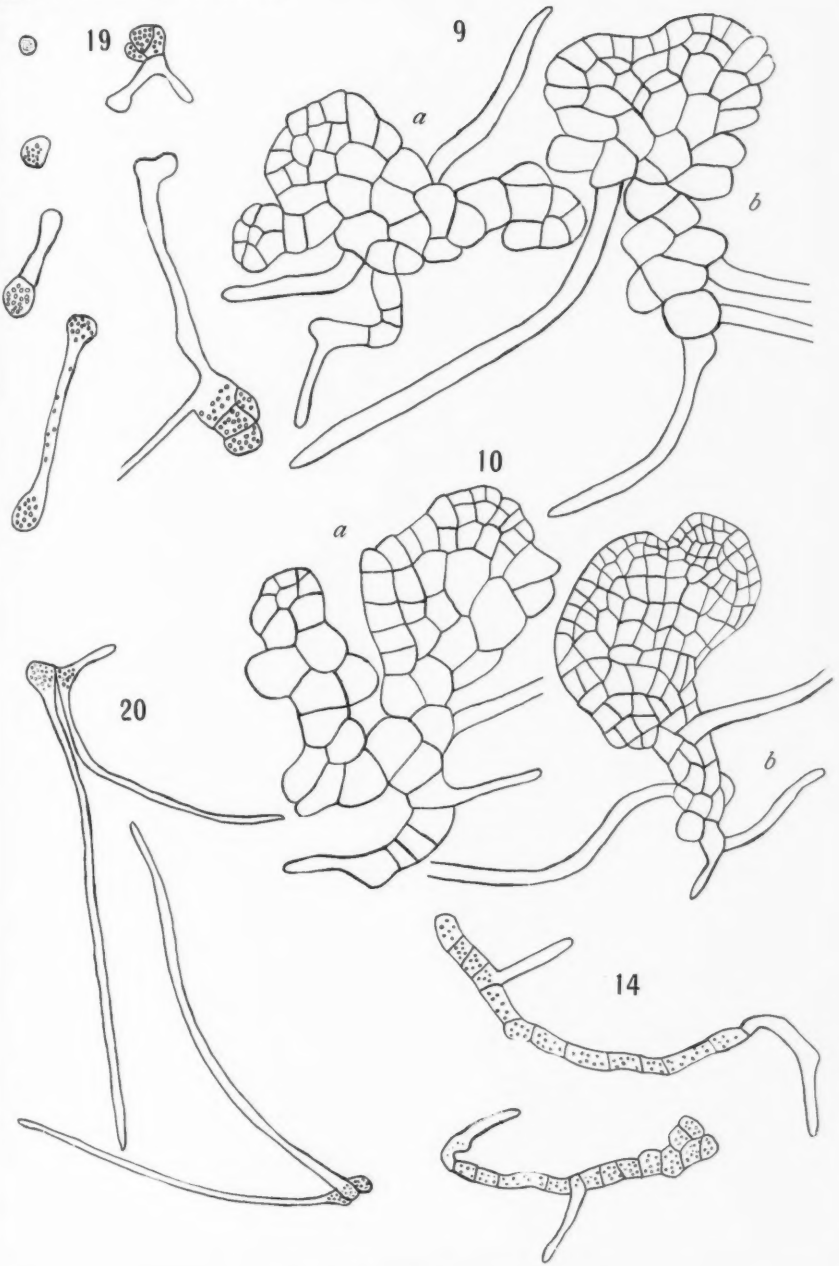
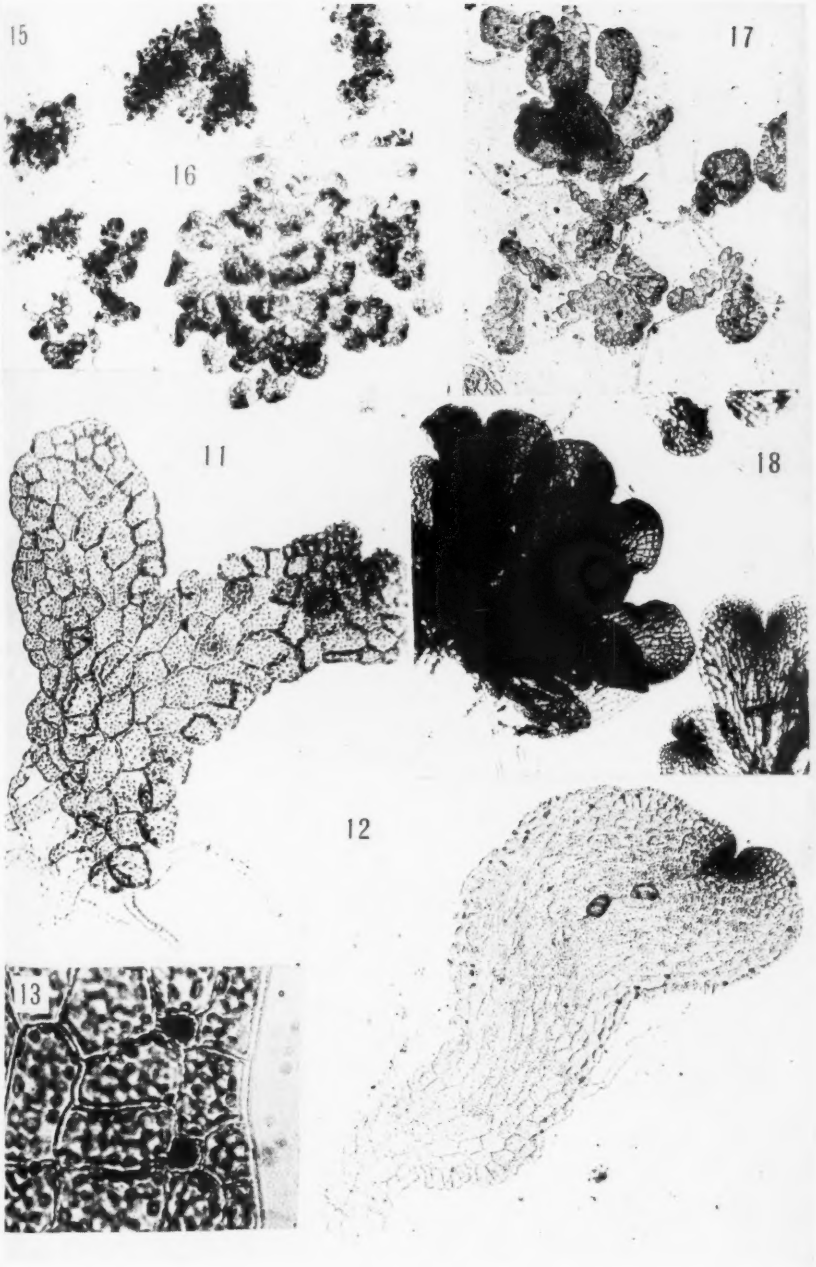


O'HANLON on MARCHANTIA



O'HANLON on MARCHANTIA

BRIEFER ARTICLES

A GENETICAL INTERPRETATION OF ECOLOGICAL ADAPTATION[1]

It is well known that different varieties of a given crop may vary greatly in their response to environmental conditions. This adaptation of a variety to more or less local variations in soil and climate is the primary reason for differences in varietal distribution in various parts of the country. Certain varieties are very local in their distribution, while others are found covering a wide range. For instance, the club wheats (*Triticum compactum*) are found almost exclusively along the Pacific coast, while Marquis wheat (*T. vulgare*) is well adapted to much of the territory in northern United States, from Washington to Maine.

Differences in response of a variety to environmental conditions are of considerable importance to the plant breeder. A variety may be developed which is well adapted to local conditions, but which is quite inferior under other conditions, even in the same general locality. The plant breeder's problem may well be illustrated by some of the work at the Maine Agricultural Experiment Station. About sixteen years ago the biologists selected a variety of sweet corn which was productive and of high quality when grown in the vicinity of Farmington, Maine. After the variety had been tested several years it was distributed to representative farmers in different parts of the state. The selected variety continued to maintain its superiority in the vicinity of Farmington, but was relatively unproductive in many localities, and in general was not as desirable as a number of other strains already established. A similar condition was found in the cereal breeding work. The Maine 340 oat, a selection from Irish Victor, is more productive and of better quality than any of the other varieties tested over a period of years. In a season which is generally unfavorable for oat production, however, Maine 340 is less productive than many other varieties. Fortunately conditions in Maine are usually favorable for very high yields of oats, and Maine 340 is very popular and widely distributed among the farmers.

Recent work with certain bean hybrids indicates that differences in adaptation to environmental conditions may be due, in part at least, to

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, no. 170.

genetic factors which are linked with factors effecting qualitative differences. If certain classes of segregates vary significantly in yield under different environmental conditions, it would seem reasonable to assume that adaptation to environment depends upon the same genetic basis as the segregation of simple qualitative factors, such as pattern and color.

A cross was made between Improved Yellow Eye (I.Y.E.) and a small white bean of local origin. The I.Y.E. is relatively unproductive, and in 1924 yielded 14 gm. per plant as compared with 25 gm. per plant for the white parent. The beans on the F_1 plants were completely pigmented and mottled, indicating that factors for complete pigmentation and for mottling were contributed by the white parent. The F_2 gave a ratio for

TABLE I

YIELDS IN GM. PER PLANT OF SEGREGATES OF CROSS I.Y.E.×WHITE

GENERATION	WHITE		SELF-COLORED		MOTTLED	
	N	Weight	N	Weight	N	Weight
F_3 1923....	478	8.90±0.16	241	8.03±0.19	655	10.05±0.15
F_4 1924....	79	45.9 ±0.17	32	39.1 ±2.01	591	39.9 ±0.6
F_5A 1925....	590	16.92±0.25	384	21.05±0.34	486	19.89±0.29
F_5B 1925....	585	26.79±0.36	384	25.17±0.41	465	30.57±0.45

27 mottled; 9 self-colored; 12 eyed; 16 white; indicating that the factors for mottling, complete extension of the pigments, and pigmentation belong to independent linkage groups. Each of these characters is dependent upon a single factor difference, and each shows a simple Mendelian segregation.

The entire F_3 was planted in such a way that the various classes of segregates were distributed at random throughout the field. The soil was not particularly well adapted for beans and the season was very unfavorable for bean production. The average yield per plant was about 9 gm. As shown in table I, the average yields per plant for the three classes of segregates differed significantly. Previous work[2] had already shown that seed size was linked with simple qualitative differences, so that the association of yield with color and pattern differences was not unexpected.[3] In F_3 the mottled segregates were significantly more productive than the self-colored ones. Since three-eighths of the white segregates

[2] SAX, K., The association of size differences with seed coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics **8**:552–560. 1923.

[3] ———, The nature of size inheritance. Proc. Nat. Acad. Sci. **10**:224–227. 1924.

should be homozygous for mottling and one-fourth should be heterozygous for mottling, this class would be expected to be, and is, intermediate in productivity as compared with the two pigmented classes of segregates. These results show that yield, to some extent at least, is dependent upon genetic factors which are linked or associated with factors for simple qualitative differences, and, therefore, are inherited in the usual Mendelian manner.

Only F_3 mottled segregates were selected for planting in F_4. Ten seeds of each F_3 plant were used. The F_4 rows were planted on ideal soil for beans, and the climatic conditions in 1924 were very favorable. The average yield of all classes of segregates was 41 gm. per plant as compared with 9 gm. per plant in 1923. Many of the F_3 mottled plants segregated into mottled, self-colored, and white segregates in F_4. The mottled segregates of course are far in excess of either the white or self-colored, but sufficient of the last two classes are available to make the comparisons of productivity. As shown in table I, the white segregates were significantly more productive than either the mottled or self-colored segregates. Since the F_4 families which were heterozygous for pigmentation and mottling factors were presumably distributed at random over the plots, there should be no differential effect due to soil heterogeneity in respect to the yields of the three classes of segregates. In order to check environmental effects of location, the white segregates were also compared with only the mottled segregates in the same row. A comparison of white and mottled classes in segregating rows gave practically the same results as a comparison of all white segregates with all of the mottled individuals.

The facts that the mottled segregates were most productive in F_3 under unfavorable conditions, and that the white segregates were most productive in F_4 under favorable conditions, suggest that the factor for high yield which is associated with the recessive pigmentation factor is effective only under certain environmental conditions, while the factor or factors for productivity which are associated with the mottling factor are effective under other environmental conditions.

If productivity is due to the same genetic factors in successive years there should be some correlation between the yield of the F_3 plants and the F_4 progeny. There is, however, no significant correlation between the yields of the F_3 plants and the F_4 segregates ($r=0.13\pm0.07$). This low degree of correlation can hardly be attributed to lack of genetic differences in productivity. The great variation in plant yield is indicated by the coefficients of variability, which are about 50 for each class of segregates in each of the three years. Much of this great variability undoubtedly is

due to environmental factors, but the fact that the coefficients of variability of the white and I.Y.E. parents for 1923 were only 21 and 20, respectively, indicates that considerable variation in yield was due to heredity. Although hereditary differences are apparently involved, there is no significant correlation between the yields of F_3 plants and their F_4 progeny, further supporting the suggestion that hereditary factors were involved which affected the differences in productivity of the various classes in successive years.

In 1925, 30 white, 30 self-colored, and 30 mottled segregates were selected from the F_4. The seeds of each plant were divided into two parts and designated as *A* and *B* in addition to the selection number. Lot *A* was planted in heavy soil not well adapted to beans, and lot *B* was planted in a better type of soil a quarter of a mile away. Approximately the same number of seeds was planted in the two selections of each F_4 plant. These classes of segregates, white, self-colored, and mottled, were not planted at random in the two plots, although an attempt was made to avoid soil differences between classes by making the plots long and narrow and planting each class of segregates in rows the length of the plot. The arrangement of pattern classes in long rows to avoid the effect of soil heterogeneity was of doubtful value in plot *A*, because when white segregates from F_4 mottled plants were compared with the mottled segregates in the same rows there was no significant difference in yield, although the white segregates from F_4 white plants are less productive than the self-colored or mottled segregates. In plot *B*, however, the mottled segregates were found to be significantly more productive than either the self-colored or white segregates. In both plots, *A* and *B*, the yields per plant are intermediate between the yields found in F_3 and F_4 (table I). The differences in productivity of various classes are not very consistent in F_5, although perhaps under a still different state of environmental conditions the intermediate yields should not necessarily mean a corresponding rank in productivity of the various classes of segregates. In F_5 the two lots *A* and *B* do not differ greatly in yield, and neither lot shows any significant correlation with the parental F_4 plant ($r = 0.20 \pm 0.07$ and 0.01 ± 0.07 respectively). There is some correlation between the two lots of F_5 plants, however, although the correlation ($r = 0.37 \pm 0.06$) is not as high as one might expect, considering that the corresponding F_5 rows should be practically identical in genetic constitution, and that the yields per plant do not differ greatly. Although there is some evidence of differential adaptation of yield factors in F_5, the results are not as consistent as those found in F_3 and F_4. Altogether, however, there is con-

siderable evidence that the effect of yield factors is dependent on environmental conditions.

The genetic interpretation of adaptation to environmental conditions is also supported by ENGLEDOW'S[4] recent experiment in spacing tests with cereals. Two varieties of wheat were planted at different distances, from 2 inches×2 inches to 18 inches×6 inches. One of the varieties, Red Fife, yielded about 24 bushels per acre under field conditions, while the other variety, Hybrid H, yielded about 40 bushels per acre. When the plants are closely spaced (2″×2″) Red Fife is more productive than Hybrid H. When the plants have a little more space (2″×6″) the two varieties are about equally productive, and at greater distances Hybrid H is more productive than Red Fife. It is evident that Red Fife is better adapted to crowding, and that Hybrid H is more responsive to increased opportunities for using the available space. Undoubtedly these differences in response to spacing play an important part in field production. It is also probable that genetic factors are involved in varietal differences of this kind.

Conclusion

In a cross of Improved Yellow Eye×Small White beans, there is a simple 3:1 segregation for pigmented versus white and for mottled versus self-colored in F_2. These differences in seed coat pattern are associated with differences in yield per plant, indicating that factors for plant yield are linked with these simple qualitative factors (it is possible of course that the same factor affects yield and seed coat pattern). In F_3, under unfavorable conditions, the mottled segregates were more productive than the self-colored or white segregates; while in F_4, when all classes were relatively productive, the white segregates were the highest yielders. This behavior suggests that the factors for yield are dependent on environmental conditions for their expression, and that ecological adaptation and distribution of economic plant varieties are dependent on genetic factors.

—KARL SAX, *Maine Agricultural Experiment Station, Orono, Me.*

[*Accepted for publication January 14, 1926*]

[4] ENGLEDOW, F. L., Investigations on yield in the cereals. Jour. Agric. Science 15:125–146. 1925.

CURRENT LITERATURE

BOOK REVIEWS

Root development

WEAVER[1] has produced a book on *Root development of field crops* that will prove of great interest and value to workers in all lines of plant science, including equally those interested in pure and applied research, as well as some practical producers. It is also sure to stimulate investigation in a field that has had far too little attention, and will interest horticulturists, agronomists, and market gardeners in a phase of their problems that has been too little considered.

The chapter headings give a good idea of the scope and organization of the book: the environment of the root; the soil; how roots are built to perform their work; root habits in relation to crop production; root habits of native plants and how they indicate crop behavior. Chapters V–XVI deal with the root habits of wheat, rye, oats, barley, corn, sorghum, various meadow and pasture grasses, sugar beets, alfalfa, various clovers, potato, and sunflower. Chapter XVII describes the methods of studying root development. The bibliography consists of 232 citations, and there is also a comprehensive index of 16 pages.

The reviewer feels that the book is a most judicious statement of the factors affecting root development and of the effect of root development upon crop production. Many factors are considered. First is the hereditary factor. The roots of some plants are very fixed in form, being modified relatively little by environmental factors. The roots of other plants are far more plastic, being greatly modified by environmental conditions. This is generally true of crop plants. The effect of the following environmental factors are rather fully considered: soil and subsoil; water and nutrients in the soil and subsoil, including irrigation of different amounts and times of application, and the application of fertilizers of various sorts to the soil and subsoil; transplanting, tillage, intercropping; rotations of various sorts; and acids, alkalies, temperatures, and oxygen supply. The literature on the effect of conditions upon the susceptibility and resistance of roots to diseases is also brought together.

While many of the data presented are from the extensive work of the author and his associates, all other sources of information have been drawn upon, including agronomy, horticulture, physiology, and pathology. The book is an excellent critical treatise on the information in this field to date. As one reads it, he wishes that every phase of plant science as bearing on practice was as well summarized and as critically treated.—W. CROCKER.

[1] WEAVER, J. E., Root development of field crops. 8vo. pp. xii+291. New York: Hill Book Co. 1926. $3.00.

MINOR NOTICES

Imperial Botanical Conference

A considerable number of the papers and discussions at the Imperial Botanical Conference[2] held at London, July 1924, took up matters of pathological and mycological interest.

It is only natural that at a conference attended by botanists from all parts of the British Empire there should be papers on the diseases which affect important crops in the British Isles, the dominions, and the colonies. The following crops were represented: apple, citrus, clove, coconut and other palms, potato, rubber, sandal, sugar cane, tomato, and forest trees and timber. Several papers showed that the marketing and storage of fresh plant food products are presenting problems in England, as in the United States, and that botanical research is being used to cope with them. Under the heading "The biological problems of the cold storage of apples," a series of papers deals with various aspects of the "ontogenetic metabolic drift" of apples in storage, and the relations of various living and non-living factors thereto. The concept of a metabolic drift should prove to be very useful.

There were several papers on the relation of plant pathology to genetics. A plea was made (Dr. WM. B. BRIERLEY) that the plant pathologists and mycologists acquaint themselves with the work of the modern geneticist, so that they may, as much as possible, use the concepts and criteria of the geneticists when they engage in genetical considerations of their problem. It is undoubtedly true that there has been too much of "thrusting results somewhat blindly into the framework of genetics."

It was pointed out that the pathologist and mycologist should proceed cautiously in employing the terminology of the geneticist, especially the term mutant, and take pains to make sure that the material in question is as pure genetically as is the homozygous material of the geneticists. It may be wholly unwarranted, for example, to assume that a single spore or hyphal tip culture is genetically pure.—GEO. K. K. LINK.

Forestry almanac

This useful compilation of facts[3] concerning our forests, edited by CHARLES LATHROP PACK, has been much enlarged since the appearance of the first edition was noted in this journal[4] two years ago. Developments during these two years have included considerable advances in the United States Forest Service, and this edition brings the history of this branch of the Department of Agriculture down to date. Among other data are included information regarding the organi-

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[2] Imperial Botanical Conference, London. July 7–16. 1924. Report of Proceedings. Ed. by F. T. BROOKS. Cambridge. 1925.

[3] American Tree Association. Forestry Almanac. Semicentennial edition. pp. xiii+348. Philadelphia: J. B. Lippincott Co. 1926.

[4] BOT. GAZ. **78**:356. 1924.

zation and activity of some 70 forestry organizations, and of over 20 college and university courses in forestry. Its list of books on forestry is also extensive. In a word, it seems to contain more information regarding forestry and forest conditions than any other book before the public.—GEO. D. FULLER.

NOTES FOR STUDENTS

Taxonomic notes.—DOWNIE[5] has published descriptions of the new orchids from Siam determined by the late R. A. ROLFE. The collections were made by A. F. G. KERR, and include 35 new species, representing 17 genera.

MERRILL[6] has published a list of plants collected on Banguey Island, which lies between Borneo and the Philippines. The collection was made by some forest rangers, and is estimated to represent probably only about one-fourth of the flora. The flora of the island as represented in this collection is remarkably intermediate between that of the Philippines and that of Borneo. More than 400 species are listed, and 21 of them are described as new. The specimens are on deposit in the Herbarium of the University of California.

ASHE[7] has published some notes on the development of pubescence in *Tilia*, and also describes a new species (*T. lata*) from Alabama. He also publishes a key to the species of *Tilia* in eastern United States with lower leaf surface tomentose at time of flowering.

OSTERHOUT[8] has described three new species from Colorado under the following genera: *Atriplex*, *Sophia*, and *Miltitzia*.

MERRILL[9] has published a second paper describing new species from Indo-China, based on material collected by A. PETELOT. It includes 18 new species, and 18 others recorded from Indo-China for the first time. The new species are distributed among 14 families.

In continuation of his investigation of Fabaceae, RYDBERG[10] has presented 2 genera segregated from *Astragalus*, namely, *Geoprumnon* Rydberg, with 7 species, and *Hesperastragalus* Heller, with 12 species, 2 of which are described as new.

NELSON[11] has presented "the loco plants," in view of their return to *Oxytropis*. He describes 9 species, one being new, presents 8 other species, and closes

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[5] DOWNIE, D. G., Contributions to the flora of Siam. Bull. Kew Gardens no. 9. 1925 (pp. 367–394).

[6] MERRILL, E. D., The flora of Banguey Island. Philippine Jour. Sci. **29**:341–427. 1926.

[7] ASHE, W. W., Notes on *Tilia*. Bull. Torr. Bot. Club **53**:27–33. 1926.

[8] OSTERHOUT, G. E., New plants from Colorado. Bull. Torr. Bot. Club **53**:35–36. 1926.

[9] MERRILL, E. D., New species of plants from Indo-China. II. Univ. Calif. Publ. Bot. **13**:127–143. 1926.

[10] RYDBERG, AXEL, Notes on Fabaceae. VII. Bull. Torr. Bot. Club **53**:161–169. 1926.

[11] NELSON, A., Taxonomic studies. Univ. Wyoming Publ. Bot. **1**:109–143. 1926.

the account by considering "troublesome pairs and doubtful species," 5 in number, 2 of which are described as new. He also describes and discusses 22 new species in 13 miscellaneous genera, 5 of which belong to *Phlox*, 3 to *Gaillardia*, and 3 to *Senecio*.

PAYSON[12] has presented 3 genera, with full descriptions, citations, and illustrations. The genus *Thlaspi* in North America is recognized as including 6 species, with one new species and 2 new varieties. *Oreocarya* includes 7 species, 5 of which are new. The third genus is considered under the title "*Erigeron compositus* and its allies in the United States," involving the presentation of 4 species (one of which is new) and 4 varieties. He has also published a long list of names that have been used for *Erigeron compositus* and its allies.

RUSBY,[13] following BITTER's reinstatement and extension of *Lycianthes* (Solanaceae), has published a number of transfers from other genera and also some new species. In BITTER's presentation, over 300 species were transferred from other genera, 250 of them from *Solanum*, so that *Lycianthes* is a very large genus. RUSBY adds 6 new species (from Bolivia and Colombia) and 5 new combinations, from material in the herbarium of the New York Botanical Garden.—J. M. C.

Bud formation.—SUMMERS[14] has summarized the literature dealing with the problem of bud formation in plants. Attention is called to the present great need in horticulture of going beyond the common horticultural practices of pruning, grafting, defoliation, and ringing, and applying the methods of the plant physiologist and the plant biochemist in determining the factors that are really modifying the activity of the plant. The review is a survey of the progress made by the plant physiologist in this field, and is organized about the following main subjects: the sap concentration factor; LOEB's inhibition hypothesis, with evidence for and against it; the food reserves of the shoot; the resting period of buds; ringing experiments; and certain miscellaneous experiments bearing on the problem of bud formation.

Of these various lines of attack, SUMMERS rightly states that the studies having to do with the food reserves and the sap concentration promise to throw more light on the problem of bud formation. The work of KRAUS and KRAYBILL, BUTLER, SMITH and CURRY, HARVEY, and others has been summarized in this section. This part of the review seems rather incomplete, although much of the work done since KRAUS and KRAYBILL's paper in an attempt to get at the chemi-

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[12] PAYSON, E. B., *Thlaspi*, *Oreocarya*, and *Erigeron*. Univ. Wyoming Publ. Bot. 1:145–186. 1926.

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cal basis of vegetation and reproduction was probably published too late to be included in the review.

While the study of the chemical situation in the plant seems to give the greatest promise of explaining the problems of correlation and bud formation, it must be admitted that much further work remains to be done before we have a satisfactory explanation of these problems. It may also well be that stimuli, hormones, and inhibiting substances will have a place when the full story is told.

MISS FERNALD[15] has published a recent paper on the subject of bud development. She finds that there is a rather close correlation between the osmotic concentration of a tissue and the tendency of this tissue to inhibit the development of other tissues or be inhibited by them. For example, it was found that a potato with a strong terminal sprout nearly always showed a higher concentration of the sap of the apical eyes than of the more basal eyes. Also shoots of privet and *Philadelphus* whose terminal buds were actively growing, and which did not develop lateral branches the first year, generally showed a more concentrated sap in the apical portions than in the more basal portions. The author realizes that while osmotic concentration may be an important factor inhibiting bud development, it is only one of the possible factors involved.—S. V. EATON.

Flora of the Black Hills.—This bulletin[16] is devoted primarily to descriptions of the geologic formations of the Black Hills and to the mineralogy and economic geology of the region. It includes, however, a section on the flora of the State Park by A. C. MCINTOSH, Assistant Professor of Biology at the State School of Mines, who has recently made extensive collections of the plants of the Black Hills, including more than 400 species from the State Park. Some 200 of these are listed under the following habitats: foothills, foothills streams, mountains, moist ravines, valleys, the gorge and granite peaks.—H. E. HAYWARD.

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